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Previous reports suggest that high dairy calcium diets help augment total and regional fat loss in obese women. Other reports suggest that timed protein ingestion before and after resistance exercise can augment lean body mass as a result of resistance training. The objective of this study was to examine both the calcium/fat loss and the protein supplement hypothesis in overweight women with chronic low calcium diets who participated in a resistance training program with calorie restriction. Participants (age = 36.6 ± 4.7; African American 57.7%, White 30.8%, 11.5% other) with a BMI of 29.1 ± 2.2 kg/m² were randomized to low calcium (LC) (≤ 500 mg; n=13) or high calcium (HC) (≥1200 mg; n=13) and yogurt (YOG) or control (CONT) supplements. All participants received reduced calorie (250 kcal deficit) diets. Six dietary recalls were obtained by a multi-pass approach provided by Nutrition Data System software. Body composition was measured by dual energy x-ray absorptiometry, waist circumference, and sagittal diameter. Participants completed 16 weeks of whole body resistance training three times per week. Mean weight loss in the total sample trended toward significance (1.9 kg; p = 0.06) and corresponded to significant caloric reduction from baseline (p = 0.001). The prescribed mean calcium intake was achieved for each study group (LC = 469.0 ± 148.3 and HC = 1297.0 ± 181.5 mg) with no significant changes in protein intake over time (LC = 0.92 and HC = 1.02 g/kg, p = 0.21). Fat mass index (LC = 12.3 to 11.0 and HC = 13.0 to12.2 fat kg/m²), trunk fat (LC = 1.74 to 1.54 and HC = 1.68 to 1.55 kg), waist

circumference (LC = 88.4 to 85.0 and HC 84.6 to 82.3 cm), and sagittal diameter (LC = 27.1 to 25.8 and HC = 25.6 to 24.4 cm) all significantly decreased over time ($p \leq 0.05$) with no group differences ($p \geq 0.37$). Total lean change (YOG = 0.9 ± 1.3 and CONT = 1.1 ± 1.0) increased significantly over time ($p = 0.001$) but not by group. These data suggest that high dairy calcium diets and pre/post-yogurt supplementation offer no added benefit in reducing fat or increasing lean indices when combined with resistance training and caloric restriction.

THE EFFECT OF A HIGH DAIRY DIET, DAIRY SUPPLEMENTATION AND RESISTANCE EXERCISE ON INCREASING LEAN BODY MASS AND DECREASING FAT MASS IN OVERWEIGHT WOMEN

by

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APPROVAL PAGE

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CHAPTER I

INTRODUCTION

The obesity epidemic is a problem that continues to plague the health of our society. According to the American Obesity Association, 64.5% of U.S. adults age 20 and older are overweight and 31% of the population is considered obese (AOA, 2006). In addition, the National Center for Health Statistics (2006) reports 61.4 % of U.S. women ages 20 to 74 are overweight while 34% are obese. Sadly, the rates of obesity are expected to climb in the future as they have in previous decades. Leaders in public health have stated that obesity is a neglected disease. According to the National Institutes of Health's 2005 budget, the obesity research funds were estimated at 440 million compared to the total NIH budget of 28.8 billion (AOA, 2006). Furthermore, NHANES III data suggest that obesity and overweight are related to serious medical conditions such as type II diabetes, coronary heart disease, hypertension, osteoarthritis, and birth defects.

We know that poor diet and limited physical activity contribute to the rise in weight gain. Women often cite time constraints as a primary cause for decreased physical activity (Brady et al. 1999; Evans et al. 1997; Zaravar et al. 1997). Diet and physical activity shortfalls can produce gaps between promoting short-term weight loss and lifelong management. These gaps may be narrowed by research designed to ascertain the ideal integration of nutrition and exercise strategies into a successful intervention

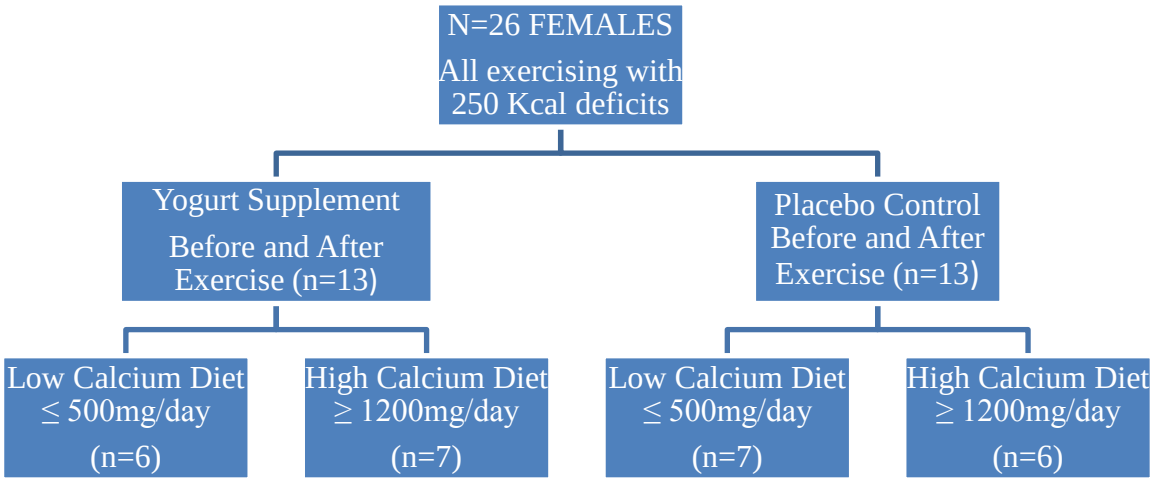
program. Future investigation in this area is critical to discover cost effective and time efficient management strategies to prevent further weight gain.

Overweight females (BMI: 25-29.9 kg/m^2) between the ages of 30 and 45 participated in this 16-week intervention. This period of adulthood is thought of as a very busy time in a person's life, often the result of greater family and career responsibilities. During this time women may lose sight of the importance of good nutrition and exercise, which may increase their chances of falling victim to overweight and obesity. We need to tackle this dilemma by examining chronic interventions that are both practical and uniquely devised for this age group. Succeeding in this endeavor will provide an exciting and cost-effective strategy designed to prevent further weight gain, improve body composition, and ultimately improve women's health by reducing their risk of chronic disease.

The encompassing aim of this dissertation was to evaluate the efficacy of chronic yogurt supplementation given before and after exercise on increasing lean body mass and enhancing hormonal response while also evaluating the efficacy of dietary calcium intakes of $\geq$ 1200 mg/day on decreasing fat mass in overweight women in two separate studies. All participants were initially randomized to receive either yogurt or placebo and further randomized into either low dietary calcium or high dietary calcium groups. Random numbers that corresponded to each of the study groups were generated by statistical software and individually placed in sealed envelopes for group assignment. All participants completed 16-weeks of whole body resistance exercise (3 times/week)

combined with a mild calorie deficit (-250 kcals/day). A pictorial of the dissertation design is provided below followed by description of each research study.

Figure 1 Cluster Randomization



This dissertation includes two separate studies involving the same sample of women derived from a clustered four group design. Each of the two studies has their individual hypotheses that were originally derived from a cluster randomized design. During the initial analysis of each individual grouping (low calcium versus high calcium or yogurt versus placebo control), we assessed the opposing grouping as a possible covariate. We did not see a confounding effect of supplement grouping on calcium group outcomes or calcium grouping on supplement group outcomes. Therefore, the studies presented did not examine the original four group design; instead they are two separate studies examining two different groupings from the same sample of participants. The objectives of this research are twofold: **Study 1:** Examine the effects of a high dairy calcium diet and resistance-training on promoting fat loss in an overweight female

sample (n =13 low calcium; n =13 high calcium). **Study 2:** Examine the effects of timed yogurt supplementation and resistance training on increasing lean body mass gains in the same sample (n = 13 yogurt versus n =13 control).The women in these studies were overweight, previously sedentary, exercised during the course of the intervention and followed diets designed to promote a modest weight loss. Both studies were conducted using the following specific aims:

Study 1

Aim 1: Evaluate the effect of a reduced calorie diet high in calcium on preventing weight gain and promoting fat mass reduction in overweight women undergoing resistance training.

Hypothesis: Participants consuming diets ≥ 1200 mg/day in dietary calcium will experience greater losses in total body fat and regional body fat compared to participants receiving ≤ 500 mg/day of dietary calcium.

The approach used to test this hypothesis was by assessing fat mass and fat-free mass via Dual Energy X-Ray Absorptiometry (DXA). Abdominal region fat changes were assessed by measuring waist circumference and sagittal diameter. We expected that healthful body composition changes will be greater in participants who consume high calcium diets. We also expected that the addition of resistance training will magnify body fat losses beyond that reported in previous calcium/weight loss studies that were lacking a controlled exercise component.

Study 2

Aim 1: Examine the role of timed yogurt supplementation in relation to resistance exercise bouts as an effective means to augment increases in lean body mass and strength.

Hypothesis: Participants chronically consuming yogurt 20 minutes before and immediately after each resistance exercise bout will experience greater increases in muscular strength and lean body mass compared to participants receiving a placebo.

The approach used to measure this hypothesis was to compare 1-repetition maximums and lean body mass measurements via DXA before and after the 16-week protocol. We expected that lean body mass and strength would be greater in participants consuming yogurt before and after each exercise bout.

Aim 2: Determine the influence of yogurt supplementation on the growth hormone, insulin growth factor-I, and insulin growth factor binding protein-3 in response to resistance training.

Hypothesis: Participants given yogurt supplementation before and after resistance training will experience larger hormonal responses compared to participants consuming a placebo before and after exercise.

The approach to measure this hypothesis was to evaluate IGF-I and IGFBP-3 fasting levels at baseline, midpoint, and endpoint while evaluating GH profiles of study participants during exercise (i.e. pre-exercise, 0 minutes post-exercise, 30 minutes post-exercise, and 60 minutes post-exercise) at the beginning and end of the 16-week resistance-training program. We expected that yogurt supplementation would promote

greater anabolic hormone responses that would positively correlate with increases in lean body mass.

The hypotheses formulated for these studies was based on the current literature supporting the role of dietary calcium in promoting abdominal and total fat loss (Zemel et al, 2004) as well as the potential benefit of protein ingestion before and after resistance exercise on facilitating amino acid uptake in skeletal muscle (Tipton et al, 2001)

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CHAPTER II

REVIEW OF LITERATURE

Calcium intake trends of women in the United States

Dairy calcium intake among women in the U.S. varies across age and ethnicity. According to NHANES III data, all females between the ages of 12 and 59 maintain mean calcium intakes between 700-800 mg/day, a level below the current recommended daily allowance for calcium for females of this age range (Fulgoni et al, 2007). When age and ethnicity are both examined, African American females consume significantly less calcium as they age and significantly lower levels of calcium compared to other age-matched ethnicities (Fulgoni et al, 2007). Furthermore, daily calcium deficits are not overcome by high rates of calcium supplementation. According to NHANES III data, only 29.7% of non-Hispanic white women and 10.5% of non-Hispanic black take calcium supplements. In addition, only 17.7% of all women between the ages of 17 and 44 report using calcium supplements.

Table 1 Average calcium intakes among women 31-50 years of age

Females 31-50 years *significantly less at $p < .05$	NHANES 99-00 (Fulgoni et al, 2007)	CSFII (Fulgoni et al, 2007)
African American Females	556.9 +/-29.2*	509.9 +/-28.2*
All other Females	768.9 +/-27.0	666.9 +/-11.6

The effect of dairy calcium on body weight and fat mass

It is suggested that calcium plays a pivotal role in reducing body fat accumulation and may function as a regulator of energy metabolism (Zemel, 2004; Zemel 2005). Two proposed mechanisms are widely accepted to explain how dietary calcium influences these changes (Parikh, 2003). The primary proposed mechanism is associated with the hormone calcitriol and its role in the regulation of intracellular calcium levels in the adipocyte (Parikh, 2003). This mechanistic theory originated with Zemel in over 21 studies examining agouti gene function in adipocyte metabolism and eventually laid the framework for future work examining dietary calcium modulation with obesity (Zemel, 2005). A second mechanism explains the role of dietary calcium in regulating adiposity (Parikh, 2003). This mechanism is explained by dietary calcium binding to fat within the gastrointestinal tract forming a poorly absorbed complex. This binding occurs with elevated calcium intake causing reduced absorption of triacyglycerol which leads to greater fecal fat excretion. The contribution of this mechanism is small (2 g supplemental calcium/day yields ~1% excretion of energy from fat/day in a 2500 kcal, 33% fat diet) (Parikh, 2003). Because of the minimal role this mechanism plays, fecal fat excretion is not the focus of this review.

To further explain the primary proposed mechanism, calcitriol regulates adipocyte calcium entry via a specific vitamin D-MARRS receptor. According to the hypothesis of Zemel (2005), an elevation of extracellular calcitriol, induced by a low calcium diet, facilitates calcium influx into the adipocyte (Zemel, 2005). This entry of calcium stimulates lipogenic gene expression and can lead to increased lipogenesis, decreased

lipolysis, and increased adipocyte lipid filling. Calcitriol also acts on a nuclear Vitamin D receptor to decrease the expression of adipocyte uncoupling protein 2 (UCP2). This leads to decreased fatty acid transport and oxidation within the mitochondria. In contrast to low calcium diets, high calcium diets appear to reduce calcitriol levels, inhibit lipogenesis, and promote greater adipocyte lipolysis, and lipid oxidation.

High calcium diets seem to have an attenuating effect on human adiposity, but high intakes of dairy seem to promote larger losses in total fat and abdominal fat than diets fortified with calcium alone (Zemel et al. 2004; Zemel et al. 2005). The cause of the additive effect of dairy calcium is still unclear but it is hypothesized that it may be related to bioactive compounds of dairy such as angiotensin converting enzyme (ACE) inhibitors and branched chain amino acids (BCAAs). These components may work synergistically with calcium or play independent roles. Furthermore, adipocytes have an intrinsic autocrine rennin-angiotensin system. Normally angiotensin II works in adipocytes to upregulate fatty acid synthase expression (Zemel, 2004). ACE inhibitors that are present in whey are thought to suppress the adipocyte renin angiotensin system by working synergistically with calcium to reduce adipocity. Causey et al. (2003) has shown that treatment with dairy derived ACE inhibitors in energy restricted mice augments the effect of supplemented calcium on fat and weight loss, but this effect was less than what was observed in energy restricted mice consuming high amounts of dairy alone.

Despite the significant effect of both calcium and ACE inhibitors on weight and fat loss, a significant portion of the dairy effect remains unexplained. BCAAs may explain a portion of this gap because of their metabolic roles as energy substrates and

regulators of muscle protein synthesis (Layman, 2003). BCAAs make up a significant portion of dairy product protein (~26%) and if consumed at levels beyond the minimum for protein synthesis may contribute to the repartitioning of dietary energy from adipose tissue to skeletal muscle (Zemel, 2005).

One additional cellular hypothesis that may help explain the dairy calcium role in regional fat loss has been suggested. It has been proposed from invitro studies that increased serum calcitriol caused in part by calcium deficiency may function to over express 11β-HSD1 and ultimately augment the production of autocrine adipocyte cortisol. In the rat model elevated adipocyte cortisol is known to increase cellular lipid content. It is therefore, hypothesized that the selective loss of central obesity from a high dairy intake may be caused in part by the regional reduction of cortisol levels in truncal adipocytes (Morris & Zemel, 2005).

There are several observational and epidemiological studies that provide data supporting the inverse relationship between dietary calcium and body weight/fat reduction across several demographic groups (Zemel, 2005). This association between dietary calcium and body weight was first seen by McCarron et al. (1983) when analyzing data from NHANES I. A statistically significant inverse association between calcium intake and body weight was discovered. Since then, Heaney et al. (2002) re-analyzed data from 6 observational and 3 controlled trials and found that for every 300 mg increment in regular calcium intake is associated with a 2.5-3 kg lower body weight for adults. Until recently, increased calcium intake has been consistently associated with decreased indices of adiposity (Heaney, 2003). In a recent cross sectional, observational

trial, Vergnaud et al. (2008) studied 6-year changes in body weight and waist circumference changes in 1022 women (mean age: 50.9, percent menopausal: 44.6, mean BMI: 23.5) as they related to calcium intake quartiles. The authors did not find a significant relationship between dairy product consumption and weight decrease in overweight or normal weight women. Surprisingly, a nonsignificant trend for increased body weight in normal weight women was observed as dairy product consumption increased (Vergnaud et al.). It should be noted that limitations of this trial include secondary data analysis, uncontrolled variation in physical activity, and no differentiation between low-fat and high-fat dairy products.

From a clinical stand point, a landmark trial by Zemel et al. (2004) confirmed the benefit of dairy calcium in reducing human adiposity both with and without calorie restriction. Some clinical trials suggest that diets high in dairy calcium and designed to maintain participant weight aid in promoting fat loss while significantly enhancing fat loss in the presence of caloric restriction (Zemel, 2005). Despite these findings, human clinical trials designed to re-examine the effect of dairy calcium on body weight and fat mass changes are still limited in number and offer equivocal results (Lanou & Barnard, 2008). The focus of this review is to examine the results of clinical trials in this area.

The first clinical relationship between fat loss and dietary calcium intake was reported by Zemel's group when studying the antihypertensive effect of calcium in obese African American males (Zemel et al., 2000). The results of this nonrandomized trial included a 4.9 kg reduction in body fat (bioelectrical impedance analysis) from baseline measures in study participants who increased their dietary calcium intake from 400 to

1000 mg per day over the course of a year by adding two servings of yogurt per day to their diet. Zemel et al. (2004) provided further clinical examination by investigating the effects of increasing dietary calcium during a calorie deficit in obese adults. Thirty-two obese adults, 18-60 years of age, were randomized to one of three calorie restricted (-500 kcal/day) diet groups: a) control diet consisting of 400-500 mg Ca/day (0-1 servings of dairy per day + placebo supplement) (n=10); b) high supplemental calcium diet consisting of 1200-1300 mg Ca/day (0-1 servings of dairy per day + 800 mg Ca supplement) (n=11); c) high-dairy diet consisting of 1200-1300 mg Ca/day (+ 3 dairy servings of dairy per day + placebo supplement) (n=11). Measurements of lean and fat mass were determined by Dual Energy X-Ray Absorptiometry (DXA). Significant weight loss was seen between all groups from baseline to endpoint and was likely the primary result of an energy deficit. However, the calcium supplemented group (8.58 kg) lost 26% more weight than control while the high dairy group (11.07 kg) lost 70% more weight than control. A significant trend ($p<.01$) of percent body fat reduction increase was noted in a stepwise fashion from low calcium to high calcium to high dairy. A similar trend was also observed when looking at percentage of trunk fat lost and waist circumference. Fat loss in the trunk region, determined by measuring waist circumference, was 19% in the low calcium group compared to 50% in the high calcium and 66% in the dairy group. Follow up work by Zemel et al. (2005) examined the effect of a yogurt diet consisting of 3 yogurt servings per day (1100 mg calcium/day) versus a low dietary calcium diet (500 mg calcium/day) on total and central fat loss. All participants were obese and between the ages of 39 and 42. Participant followed a 500 kcal/day energy deficit diet. Results were

similar to Zemel's 2004 trial that found significant losses in trunk and total body fat among the high dairy group. In this yogurt supplementation trial, Zemel et al. (2005) found that participants consuming three six-ounce servings of yogurt per day lost 4.43 kg of body fat compared to 2.75 kg in the control group ($p<.005$). DXA measurements revealed a 1.74 kg loss in trunk fat among controls compared to a 3.16 kg loss in the yogurt group ($p< .01$). Waist circumference reduction was significantly greater in the yogurt group (-3.99 cm) versus the control (-0.58 cm) ($p< .001$). In addition, losses in lean body mass associated with weight loss were reduced by 31% in the yogurt group. To confirm these findings, Zemel et al. (2004) conducted a multi-center trial (N=105) and results were similar to previous findings suggesting that high dairy consumption created approximately a two-fold decrease in trunk and total fat compared with controls. However, in contrast to Zemel's initial findings that suggested a fat loss effect with calcium supplements, the multi-center trial did not observe a significant effect with supplemental calcium. The lack of a supplemental calcium effect may be due to the absence of bioactive compounds (ACE inhibitors, BCAA's, etc.) that are abundant in dairy products but are absent in calcium supplements. Zemel et al. (2005) added to this work by studying obese African American adults, 41-43 years of age, in two separate six-month trials. One trial featured a caloric deficit (500 kcal/day) while the other provided adequate calories for weight maintenance. Each trial featured two study groups consisting of either a low (500 mg) or high (1200 mg) calcium intake. In the weight maintenance trial, participants who consumed high dairy lost significantly more body fat (-2.16 kg vs. -.17 kg) and trunk fat (-1.03 kg vs. -.367 kg) than participants on a low dairy diet. High

dairy group participants also experienced a significant waist circumference reduction while the low dairy group did not. In the second trial (500 kcal deficit), both groups produced significant fat and weight losses. However, the high dairy diet produced approximately twice the percent decrease in body fat and weight loss compared to the low dairy group. In addition, the loss in lean body mass associated with weight loss was much less (-148 g reduction) in the high dairy group compared to the low dairy group (-1988 g reduction). The results from these clinical studies remarkably indicate that a high dairy diet clearly increases fat loss secondary to small energy restrictions and appears to play a selective role in the reduction of central obesity (Zemel, 2005).

In contrast to Zemel's work, Thompson et al. (2005) did not find a significant effect of dietary calcium on fat loss in obese participants. In this trial the authors examined the effect of a high dairy calcium diet (1400 mg), a high dairy calcium diet (1400 mg) with high fiber and low glycemic characteristics, and a standard diet (800 mg calcium) on fat and weight loss. Seventy-two obese participants were randomly assigned to one of the three experimental diets (each with a 500 kcal/day diet deficit). Participants were asked to engage in aerobic exercise 30 minutes 4 times per week during the course of the study. Participants were studied for 48 weeks while monitoring weight changes and fat/trunk mass loss via DXA. All participants' lost significant amounts of weight and body fat, however the changes that occurred were independent of calcium intake or alterations in total fiber and glycemic load. There were no statistically significant differences between groups after examining weight, fat mass, trunk fat, waist

circumference, and hip circumference. The lack of significant effects may be secondary to the high and nearly optimal calcium intake (800 mg) of the study controls.

In agreement with these results, Shapses et al. (2004) studied 100 pre-menopausal and post-menopausal women with a BMI range of 28-42 in a randomized, placebo controlled, double blinded study to determine the effects of calcium supplementation (1 g/day) versus placebo on weight and fat loss during daily 500 kcal dietary deficits. This trial pooled results from three different 25 week long studies and found no effect of supplementation on fat or weight loss, but small trends toward significance were reported. The study also found no supplement and menopausal interactions. Limitations of this study may have affected study outcomes. For example, we know that calcium supplements lack the bioactive components present in dairy foods that have been previously cited as contributors to fat loss (Zemel et al. 2004; Zemel et al. 2005). Shapses et al. (2004) also reported baseline calcium intakes ranging from 600-1019 mg/day and created total sample diet discrepancies by including a subset of participants that used replacements (Slim Fast) for the first eight weeks of the study intervention.

Bowen et al. (2005) also reported that dietary calcium did not significantly affect weight loss and body composition. Fifty overweight and obese men and women, mean ages 46 and 49 respectively, were randomized to receive a diet high in dairy protein and calcium (2400 mg) or a mixed protein, low calcium (500 mg) diet. All participants followed a 12-week calorie restriction period (promoting an energy deficit) followed by 4 weeks of energy balance. Reported intakes revealed that the dairy protein diet provided an average daily energy deficit of 279 kcals while the mixed protein diet provided an

average daily energy deficit of 428 kcals during the energy restriction phase. The authors measured weight, changes in body composition via DXA, as well as changes in markers of cardiovascular risk. Both diets produced significant weight (-9.7 kg) and fat losses (-8.3 kg), but differences were reported by the authors to be independent of the diet type suggesting that dietary calcium plays a minimal role. The authors also noted that observed changes were not influenced by protein source.

When interpreting these findings, it is important to consider the differences in calorie restriction between groups along with the fact that females in the dairy protein group were found to show a trend toward greater weight, total fat and abdominal fat losses when compared to the mixed protein females. In addition, there was a large difference in participant protein intake in this study by Bowen (2005) (34%) when compared to Zemel's clinical trials (18%). This is important because some studies have shown greater weight losses with high protein diets and the overall weight loss seen in Bowen's 2005 work was nearly 2-fold greater than what was seen in Zemel's trials. The effect of a high protein diet in Bowen's 2005 work may have hid the presence of any calcium effect. Finally, baseline calcium intakes in Bowen's 2005 study ranged from 737-935 mg compared to Zemel et al. (2004) in which baseline levels were reported as $\leq$ 600 mg. This appears to be a critical observation if the goal is to examine the effects correcting a true calcium deficiency on body composition. Adding calcium to diets that are only marginally deficient may not allow the observation of appreciable body composition changes in overweight/obese participants. It is possible that near normal

calcium intakes at baseline may have hid any true experimental differences that may have been seen in participants who normally consume ≤ 600 mg/day of calcium.

This limitation is also present in a study by Gunther et al. (2005) who studied normal weight healthy women, between the ages of 18 and 30 (N=155) with average calcium intakes ~700 mg prior to study initiation. In this randomized one-year intervention, participants were placed in one of three groups and were told to maintain their normal daily caloric intake. The control group maintained their usual dairy intake while the medium and high dairy groups were instructed to increase their dairy consumption to achieve calcium intake goals of 1000-1100 mg/day (medium dairy) and 1300-1400 mg/day (high dairy). Actual reported intakes of calcium in the control, medium, and high groups were 742, 1026, 1131 mg calcium/day respectively ($p < .0001$). The authors reported no significant changes from baseline to endpoint in body weight or fat mass but did note that a trend in weight loss was present. Baseline dairy calcium intakes of ≥ 700 mg/day and intervention calcium intakes of ~ 1100 mg/day in combination with normal weight status may have compromised the ability to detect appreciable changes in body weight and fat mass.

Ochner and Lowe (2007) were the first to report weight loss maintenance and energy intake data related to calcium intake. Obese women (N=103) were recruited after completing a non-related eight week weight loss trial (mean loss = 9.66 kg). The participants were randomized to one of three study groups and monitored for 18 months post-weight loss intervention: 1) cognitive behavior therapy (CBT) for weight loss; 2) CBT plus food monitoring training; 3) CBT, food monitoring training, and reduced

energy density eating training. Five day food records and food frequency questionnaires were collected post-intervention, at 6 months and 18 months. The authors found that calcium and energy intake were correlated ($r = 0.32$; $p = 0.03$), but neither variable was a significant predictor of weight regain over the course or the follow up intervention. The authors also noted that from the 6 to 18 month follow up period there was a significant inverse relationship between calcium intake and weight gain when energy intake was controlled and higher energy intake significantly predicted weight gain when calcium intake was controlled. The authors concluded that dietary calcium may slow the progression of gaining weight by reducing the effect of greater energy intake (Ochner & Lowe, 2007).

In a study to further examine the role of dairy in weight maintenance, Zemel et al. (2008) conducted a nine month dual-site randomized trial consisting of two phases: weight loss (months 1-3) and weight maintenance (months 4-9). Eligible participants following the weight loss phase (lose greater than 10 kg or 10% of body weight, N = 270) were randomized to either low dairy (LD, <1 serving of dairy per day) or recommended dairy (RD, >3 servings of dairy per day). Primary outcome measures were body weight and body composition (DXA) measures of total and trunk fat. Secondary outcomes were diet (3-day food records measured at BL, 3, 6, and 9 months), physical activity (moderately vigorous measured in steps), and energy intake. Calcium intake during the weight loss phase was not significantly different (LD = 707 mg ± 230 vs. RD = 731 mg ± 251) but was significant during the maintenance phase (LD = 579 mg ± 166 vs. RD = 1325 mg ± 254) No overall body weight or body composition group changes were

observed between dairy groups during the maintenance phase, however a site difference was observed as LD participants at the University of Kansas study site maintained fat and body weight, while LD participants at the University of Tennessee increased fat and body weight. The RD group consistently consumed more energy than LD at the start of the maintenance phase, month 6 and at month 9 ($p < .05$) with group differences from 3 months to 9 months in respiratory quotient compared to LD (RD = 0.74 ± 0.05 to 0.76 ± 0.06; LD = 0.74 ± 0.04 to 0.77 ± 0.05; $p < 0.01$ two sample t-test). Consistent with Ochner and Lowe, 2007, the authors concluded that following a recommended dairy intake (> 3 servings/day) may allow for higher fat oxidation rates that might allow for greater energy intake without weight gain. Data from these studies suggest at least a marginal effect of dairy intake on fat oxidation, however the philological significance of the small RQ changes reported by Zemel et al. (2008) are dubious despite statistical significance.

It is important to note that limitations exist in all trials that examine the effect of dietary calcium on weight and body composition changes. As with most studies that control for diet there are concerns regarding diet compliance over the course of a trial. In addition, the baseline activity level of participants and the amount of physical activity throughout the study protocols are often unclear or not controlled for. It is possible that participants may increase physical activity over the course of the trial in order to maximize weight loss. Furthermore, there are limitations present in studies suggesting that calcium has no effect on weight loss. Many of these studies were originally designed to examine bone mineral content and conclude from secondary outcome data that calorie

restriction can produce similar weight loss regardless of calcium intake. The potential concern with making generalizations from bone studies is that participant positioning during scanning might not have been ideal for soft tissue analysis, an essential component for accurate fat tissue assessment.

Jensen et al. (2001) studied obese participants on a standard low kcal diet (1028 kcal/day maximum) and supplemented with placebo or 1000 mg calcium per day. The authors found no significant differences between groups in weight loss after 3 months. This study was designed to evaluate bone mineral content changes with weight loss and did not measure dairy intake and failed to provide individualized kcal restrictions or control for physical activity.

Farnsworth et al. (2003) determined the effect of reducing calorie intake and manipulating the daily intake ratio of carbohydrate to protein on body composition, glycemic control, and lipid concentrations in obese participants. The authors found no significant differences in weight loss between the high protein group (27% of kcals/ 1600 mg calcium) and the standard protein group (16% of kcals/ 600 mg calcium). Evaluating the role of calcium on body composition was not the primary objective of this study therefore; it is hard to generalize these findings when individual protein and energy intake is not controlled.

Two recent trials have examined the calcium and weigh/fat hypothesis while including an exercise component in their intervention. Wagner et al. (2007) studied how different forms of calcium can impact body weight and bone as a result of a 12-week weight loss trial that included daily 500 kcal deficit diets and combined resistance

training and aerobics three days per week for 45-60 minutes/session. In this randomized controlled double blinded study, premenopausal women (n=58) were placed in one of four groups: calcium lactate (1452 mg ± 301), calcium phosphate (1566 mg ± 250), placebo (788 mg ± 175), or skim milk (1514 mg ± 225). All groups lost a significant amount of weight and percent fat (measured by BIA) over the course of the intervention ($p < .01$). However, the milk group lost significantly less fat than placebo ($p < .05$) while no group differences in calorie intake were observed (Wagner et al. 2007). This study suggests that the major effect on weight and fat loss was due to exercise and includes limitations such as no measurement of usual calcium intake prior to study intervention and the relatively high calcium intake of the placebo group compared to trials that show a calcium effect (Zemel et al. 2004; Zemel et al. 2005).

White et al. (2009) conducted an eight week resistance training trial with previously untrained women (N=35, aged 18-35) randomized into one of three treatment groups: 1) Y-3 servings of yogurt per day including one yogurt immediately following training sessions, 2) PRO-low habitual calcium intake plus post-exercise isocaloric/isonitrogenous commercial gel supplement (Accel Gel), or 3) CHO-low habitual calcium intake plus post-exercise isocaloric commercial carbohydrate supplement without protein (Clif Shot). Baseline versus endpoint outcome measures included strength (1-RM), body composition (hydrodensitometry), resting metabolic rate (RMR), fat oxidation, measurements of calcitriol (25OHD), and diet (3-day food records). All groups increased FFM and strength, while decreasing percent fat over time. No group differences were observed with percent fat, strength, fat free mass, RMR, or fat

oxidation. In an interesting finding, the yogurt group was able to significantly decrease percent body fat over time (BL 32.8 ± 7.2 to 31.0 ± 6.6) with no group differences in protein/kg intake coupled with significantly higher intervention kcal intake ($p<.05$). The short length of this trial (8 weeks) along with a significantly higher mean body weight at baseline in the yogurt group are strong limitations in this trial that not only minimize body composition changes over time but also limit our ability to make generalizations regarding group comparisons.

Because of the equivocal results seen in several trials, the role of dairy in weight loss and body composition needs further examination, especially those that employ monitored and structured physical activity. It is worth reexamining the combined effect of structured resistance training and high intake of dairy calcium for maximizing fat loss and body composition changes.

Calcium and lipid oxidation

The acute effect of dietary calcium manipulation on changes in lipid oxidation by indirect calorimetry has been examined. Melanson et al. (2003) studied 35 normal weight adults in a cross-sectional design to determine if total calcium intake and calcium from dairy sources correlate with whole-body fat oxidation in a 24-hour period. Prior to participating in the trial, usual calcium intake was measured with a four day food diary. All participants completed a 24-hour whole room indirect calorimetry measurement. Acute (24 hour period) physical activity and diet were controlled during the 1-day assessment. The authors found that acute calcium intake was significantly correlated with increased rates of whole body fat oxidation over 24 hours ($p=.03$) and during sleep

(p=.04). However, after controlling for dietary fat intake, acute calcium intake explained only 10% of the variance in increased fat oxidation seen over the 24-hour period. Chronic calcium intake from the food records did not correlate with fat oxidation or respiratory quotient (RQ). The authors proposed that the acute effects seen in this trial can be attributed to the non-genomic effects of calcitriol.

Crossover trials designed to measure several days of calcium manipulation prior to observing RQ and lipid oxidation show varied results. In a follow up study, Melanson et al. (2005) examined a high dairy calcium (1400 mg) diet versus a low calcium diet (500 mg) on macronutrient oxidation for one week. Participants acted as their own control in a crossover design that also featured a caloric deficit phase (-600 kcal/day) and a balance phase. The energy deficit phase was created with both diet and physical activity modification. A novel finding of this study was that calcitriol levels declined ~10% as a result of the dairy based high calcium diet. During the energy deficit, 24 hour fat oxidation increased significantly when participants consumed a high dairy calcium diet (-136 g fat/24 hr) compared to low dairy calcium (-106 g fat/24 hr). There was no significant effect of diet treatment on RQ or 24-hour macronutrient oxidation when energy was balanced. The authors theorized that increased lipolysis was induced by the reduction of calcitriol and fat oxidation was enhanced by the ensuing exercise. Since differences in fat oxidation were not seen among diet treatments when energy was balanced, the authors concluded that the increase in fat oxidation seen during the energy deficit was primarily dictated by the increase in physical activity. However, when specifically examining the energy deficit treatment, increased fat oxidation with the high

dairy diet (HD = -136 g/day versus LD = 106 g/day) may have theoretically occurred because of enhanced dairy induced nutrient repartitioning.

Boone et al. (2005) studied twelve men in a crossover design examining three diets: high calcium/high dairy, high calcium/low dairy and low calcium/low dairy. The objective of this study was to see if energy, substrate metabolism, and adipocyte mRNA can be altered by varying calcium intakes. The authors found that seven days of each diet type did not alter substrate metabolism, energy metabolism, or gene expression of proteins associated with fat metabolism. However, serum calcitriol levels significantly rose over time in the low calcium/low dairy group when compared to the diets containing higher calcium levels ($p < .05$). Jacobsen et al. (2005), using a similar crossover design, also found no significant results when studying the effect of three different diets: low calcium (500 mg)/normal protein (15% energy); high calcium (1800 mg)/normal protein (15% energy); high calcium (1800 mg)/high protein (23% energy) on 24-hour energy expenditure and fat oxidation. However, it was interesting to note that the experimental group with normal protein and high calcium intake experienced a 2.5 fold increase in fecal fat excretion. This effect was likely caused by an interaction between calcium and dietary fatty acids resulting in the formation of insoluble fatty acid soaps. The attenuation of fecal fat excretion by the high protein diet (23% of kcals) was likely caused by protein blocking calcium/fatty acid soap formation.

In a recent study by Cummings et al. (2006), the authors examined the acute effects of varying sources of dietary calcium on post-prandial thermogenesis, lipolysis, and fat oxidation. Eight subjects participated in a 3-way crossover design. Subjects

received a breakfast meal consisting of either low dairy calcium and low vitamin D, high non-dairy calcium and low vitamin D, or high dairy calcium with vitamin D. Study measurements were taken hourly over a 6-hour period. Plasma free fatty acid concentration significantly decreased from baseline in both groups receiving high calcium meals ($p<.035$). In addition, fat oxidation rates rose significantly over time in the two calcium groups ($p<.005$). The authors concluded that regardless of the calcium source, dietary calcium actively stimulates post-prandial fat oxidation and decreases plasma free fatty acid concentration after meals.

In a trial by Teegarden et al. 2008, the authors sought to examine the impact of dietary calcium and dairy intake on total energy expenditure (TEE), the thermic effect of a meal (TEM) and fat oxidation during a weight loss trial. Overweight women ($N = 24$, 18-31 years old) were randomized into one of two study groups that both featured a 500 kcal diet deficit: 1) Placebo (< 800 mg/day dietary calcium intake), 2) 900 mg/day calcium supplement (added to diet), or 3) three servings of dairy products (adds 900 mg calcium to diet). TEE was not affected by group assignment, however fat oxidation did increase in the calcium supplemented group compared to placebo after adjusting for fat-free pass (1.5 g/hour vs. -0.6 g/hour; $p = 0.02$). Additionally, a positive correlation was observed between measured 25-OHD and TEM ($R = 0.31$, $p = 0.004$) and trended toward fat oxidation ($p = 0.06$). Finally, changes in parathyroid hormone concentration were linked with changes in trunk fat mass ($R = 0.27$, $p = 0.03$). The authors concluded that fat oxidation is enhanced by calcium intake independent of TEE while postulating that vitamin D status may enhance TEM and fat oxidation.

The acute effects of calcium intake on factors such as 24-hour energy expenditure and fat oxidation appear to be modest at best when comparing 7 day crossover diet trials. Despite the absence of a large effect, there appears to be at least a small amount of variation explained by calcium intake. The primary proposed mechanism of reducing serum calcitriol and increasing fecal fat excretion likely helps explain the benefits of dietary calcium on weight and fat loss. However, confounding factors such as physical activity, protein intake, and near normal calcium intakes reduce our ability to confirm the exact independent contribution of calcium in weight and fat loss.

Timing of protein intake and muscle protein synthesis

Recent studies have provided data to suggest a beneficial effect of timed protein ingestion before and after resistance training on factors such as improved protein synthesis (Borsheim et al. 2004; Tipton et al. 2004; Cribb & Hayes 2006) and improvements in muscle fiber strength and size (Andersen et al. 2005; Esmarck et al. 2001). Tipton and Wolfe (2004) suggest that along with considering daily protein needs and composition of protein source, the timing of ingestion is a key factor influencing muscle synthesis and function. Although not the focus of this review, it is important to note that carbohydrate ingestion immediately following resistance training also influences muscle physiology by increasing glycogen stores (Ivy 2001; Conley & Stone 1996) and decreasing protein degradation (Roy et al. 1997). Carbohydrate ingestion immediately following resistance exercise may work synergistically with protein ingestion to chronically enhance muscle mass and strength gains. For the purpose of this

review, the most relevant acute and chronic protein timing studies are presented below in detail and an overview of studies are presented in a subsequent table.

Rasmussen et al. (2000) were one of the first to report increased muscle protein synthesis by increasing amino acid availability within three hours post-exercise. The authors specifically measured muscle protein synthesis following supplementation of an amino acid-carbohydrate drink both one and three hours post-resistance exercise. Six recreationally active volunteers (3 male; 3 female) were randomly assigned to receive either the essential amino acid drink (EAA) or placebo (Pla). Each participant was studied twice and acted as both treatment and control. The EAA drink consisted of 6 g of essential amino acids and 35 g of sucrose. The Pla drink was artificially flavored and calorie free. Leg protein kinetics was determined using a 3-compartment model including muscle biopsies, phenylalanine infusion, and venous/arterial femoral blood sampling. After fasting, participants were started on a venous infusion of urea 3 hours prior and a phenylalanine/alanine mix 2 hours prior to exercise. At the time of exercise, strength tested participants completed a 1-time leg resistance training bout consisting of leg press and leg extensions at 80% of 1-RM for approximately 45 minutes. After the exercise ceased, study drinks were administered while blood samples and muscle biopsies were taken at various time points. Phenylanine net balance across the leg was significantly increased above placebo and pre-drink values ($p<.05$) after consuming the EAA drink 1 hour and 3 hours post-exercise. Results from the 3-compartment model showing muscle protein synthesis revealed significant increases (~400%) above pre-drink levels ($p<.05$) when the drink was consumed at 1 hour and 3 hours post-exercise. No significant

changes occurred with placebo or between drink administrations. The authors concluded that EAA and carbohydrate given both at 1 hour and 3 hours post-exercise increased muscle protein synthesis. Induction of protein synthesis following an EAA/CHO drink appears instantaneous and is likely the combined result of essential amino acid availability and hyperinsulinemia.

Tipton et al. (2001) was the first to report the pre-exercise effect of amino acid-carbohydrate supplementation on promoting a favorable anabolic environment for muscle growth. Six volunteers participated in a crossover design in random order with each participant consuming 500 ml of a solution containing 35 g of sucrose and 6 g of essential amino acids either immediately before (PRE) or after (POST) intense (80% 1-RM) leg resistance exercise. An artificially sweetened placebo was given either before or after exercise opposite of the experimental drink. Phenylalanine concentrations across the leg and muscle biopsies were taken at various time points from 60 minutes pre-exercise to 120 minutes post-exercise. As expected, blood and muscle phenylalanine concentrations were increased following supplementation and amino acid concentration increased from exercise in both trials. Net uptake of phenylalanine was 160% greater in the PRE-trials over the time course. Percent phenylalanine uptake was nearly threefold higher in PRE-exercise (21%) compared to POST-exercise (8%) ($p = .01$). Final calculations revealed approximately 180 mg of phenylalanine incorporated into protein (PRE) versus 39 mg (POST) ($p = .02$). The results of this study suggest that ingestion of an essential amino acid-carbohydrate beverage prior to resistance exercise elicits greater net protein synthesis compared to giving the same supplement in the post-exercise time period. The

explanation to these findings is related to Rasmussen et al. (2000) in that increased amino acid availability from supplementation contributed to favorable leg protein anabolism. In addition, increasing amino acid availability during optimal blood flow (PRE) may optimize amino acid uptake in the muscle.

Until recently no studies had examined the response of acute muscle protein metabolism from amino acids and carbohydrate found in whole food. Elliot et al. (2006) sought to determine net muscle protein balance after resistance training from the ingestion of milk. Volunteers were assigned to one of three milk groups to consume after exercise: fat free (FM) 237 g, (n=8); whole milk (WM) 237 g, (n=8); or isocaloric fat-free (IM) 393 g, (n=8). Volunteers were instructed to consume their assigned beverage one hour after completing 10 sets of leg extensions at 80% of their 1-RM. Muscle biopsies and blood samples were taken at various time points from 60 minutes prior to exercise to 300 minutes post-exercise. Milk ingestion did not alter intracellular amino acid concentration and there were no significant differences between milk groups with regard to peaks in serum phenylalanine following milk ingestion. Net threonine and phenylalanine balance over time peaked between 10 and 30 minutes following milk ingestion and was significant from baseline levels ($p \leq .001$). Significant peaks above baseline were also seen for both amino acids at and beyond 120 minutes post-ingestion. Phenylalanine and threonine exchange indicated as area under the curve for net balance was significantly greater than zero for phenylalanine ($p < .05$) and threonine ($p < .01$). Phenylalanine and threonine uptake were greater in WM and IM than in FM. The results suggest that ingestion of milk following resistance exercise helps augment muscle protein

synthesis. Previous work from the same lab group (Tipton et al. 2004) used a similar protocol and did not find a positive net balance when supplementing with a placebo. This suggests that the positive net synthesis seen in these studies were due to the anabolic effect of timed milk or milk protein ingestion which may additionally be explained by a concomitant decrease in muscle degradation. It was surprising that amino acid uptake was highest in the WM group. It was theorized that the difference may be due to slight variation in kcal density, but it is important to note that insulin response was not significantly different between groups (Elliot et al. 2006). This study provides practical application by suggesting that milk ingestion post-exercise may be an effective alternative to dietary supplements in promoting muscle protein synthesis.

Beelen et al. 2008 sought to examine the effect of protein coingestion with carbohydrate during acute whole body resistance exercise and concluded that even in a fed state (2 hours post- standardized dinner), protein ingestion during resistance exercise is beneficial in further augmenting muscle protein synthesis rates. Ten recreationally active males participated in this double blind, randomized, crossover trial. Participants received standardized diets on test days and received boluses of their assigned beverage every 15 minutes at a dose of 1.5 ml/kg during the 2-hour exercise session. The beverages were 50% glucose/50% maltodextrin with or without 0.15 g/kg/hr protein hydrolysate. Protein kinetics data from 120 minutes of post-exercise plasma sampling and muscle biopsies revealed that protein coingestion lowered protein degredation by 8.4% ($p = 0.06$) and increased synthesis rates by 33% ($p < 0.01$). In addition, whole body net

protein balance was negative in the carbohydrate only group and positive with protein coingestion (-4.4% vs. 16.3%; $p < 0.01$).

In an effort to examine if timed soy protein ingestion promotes muscle protein accretion by a similar magnitude compared to milk proteins, Wilkinson et al. (2007) provided 18 g of a soy beverage or 18g of isocaloric milk beverage before and after an acute exercise session in healthy, trained young men (N = 8). With arterio-venous blood sampling 180 minutes post-exercise the authors discovered that both beverages produced a positive net protein balance with the area under the net synthesis curve significantly higher in the milk group ($p<.05$) The authors concluded that although both protein sources promote synthesis, the milk protein may contribute to more rapid lean mass accrual over time. To test for differences in these protein sources in a chronic trial, Hartman et al. (2007) reported that milk consumption given immediately after exercise and again 1-hour post-exercise significantly contributed to greater Type II muscle fiber area compared to isonitrogenous/isocaloric soy beverage. In addition to these findings, Type I fiber area increased in both protein groups, but the milk group was the only group to create a significant increase in fiber area compared to the maltodextrin control ($p<.05$). The protocol included health young non-trained men who were engaged in 5 days per resistance training lasting 12 weeks while maintaining similar caloric and protein intakes between groups.

In a recent trial by Bird et al. (2006), 32 untrained physically active young males participated in a whole body resistance exercise bout to determine the effects of a supplemental beverage on hormone response and myofibrillar protein degradation.

Volunteers were matched and randomized into one of four supplemental groups: 6% CHO solution, 6g EAA solution, combined EAA/CHO solution, and placebo. Volunteers received 8.5 ml/kg of their assigned supplements immediately following the exercise bout. Eight exercises were included in the protocol and were performed at approximately 75% of a participant's 1-RM. Each exercise was performed with 3 sets of 10 repetitions and 1 minute rest periods. 3-methylhistidine (3-MH) was assessed by urine sampling on separate days pre-exercise and post-exercise while diet was controlled. Blood collection took place every 15 minutes during exercise and immediately post-exercise. Both 6% CHO and the EAA/CHO solution decreased cortisol concentrations 11 and 7% respectively from baseline while the placebo group experienced a significant 105% increase increase in cortisol from baseline. The changes in cortisol concentration appeared to coincide with urine 3-MH concentrations. The EAA and CHO beverages independently decreased 3-MH in a non-significant fashion. Interestingly, the combined supplement decreased 3-MH by 27% ($p<.01$) while the placebo group experienced a 51% increase in 3-MH suggesting elevated protein degradation. The authors concluded that EAA/CHO intake following resistance training may have an anti-catabolic effect by attenuating myofibrillar protein degradation.

In order to shed light on the mechanistic nature of muscle protein synthesis due to resistance exercise during a postprandial state, Oliver et al. 2009 studied 10 untrained males following a lower extremity exercise after consuming a standardized breakfast meal. Muscle biopsies coupled with arterio-venous phenylalanine infusion was used to gauge muscle synthesis rates. The authors reported an increase in S6 and 4E-BP1

phosphorylation that were significantly ($p<.05$) higher in the exercised legs versus nonexercised legs in the fed state. A similar increase in phosphorylation was not seen in S6K1 or mTOR proteins.

To date there are few chronic studies that specifically examine the importance of protein intake timing in relation to resistance training outcomes such as muscle synthesis and strength.

In the only chronic supplementation and resistance training study involving women presented in this review, Holm et al. 2008 studied the response of a post-exercise supplement on muscle mass, strength and bone formation in post-menopausal women. Twenty-nine participants completed 24 weeks of resistance training and were assigned to either a nutrient group (n = 13; 730 kJ supplement with 10 g protein, 31 g carbohydrate, 5 μg vitamin D, 250 mg calcium post-exercise) or control (n = 16; 102 kJ supplement with 6 g carbohydrate and 12 mg of calcium post- exercise). Participants engaged in a multi-phase resistance training program occurring three times per week while following calorie and protein controlled weight maintenance diets. No food intake was allowed in two hour window before and after each training bout. From week 6 to 24 there was a significant increase in isokinetic strength (measured via dynamometer) in the nutrient group compared to control (+9% versus +1%; $p < 0.05$). Both groups increased whole muscle hypertrophy and muscle fiber cross-sectional area without group differences. Fat mass changes were not observed over time or by group. Lean mass only increased in the nutrient group (42.3 to 43.1 kg; $p < .05$). L2-L4 BMD was the only bone measurement to increase over time without group differences. Finally, osteocalcin measures revealed a

significant interaction of bone formation at 24 weeks in the nutrient group compared to control. The authors concluded that post-exercise nutrient supply is essential to musculoskeletal maintenance during 24 weeks of resistance training.

Esmarck et al. (2001) examined the importance of the timing of protein intake following exercise during a period of resistance training in elderly men. Thirteen volunteers completed a 12 week resistance training program with strength measurements conducted at pre-training, mid-training, and post-training. The training program included progressive bilateral resistance exercises conducted 3 times per week. The exercises used were compound lifts and included the leg press, lat pull down, and knee extension. DXA scanning, food records, and cross-sectional muscle area were all assessed at pre-training and post-training. Participants were matched in pairs based on body composition and daily protein intake and randomly assigned to one of two study groups: P0 ($n = 7$) protein supplemented within 5 minutes following resistance exercise; P2 ($n = 6$) protein supplemented 2 hours post-exercise. The protein supplement was in the form of a gel, consisted of 10 g protein, and contained approximately 100 kcals/serving. Strength measurements included isokinetic strength measured by a dynamometer and dynamic training strength using a value of 5 RM. Muscle cross-sections were assessed with magnetic resonance scanning and muscle biopsy. Chemical analysis included an assessment of ATPase histochemistry, assessment of myosin heavy chain expression, and acute insulin response. Changes in body composition were measured with DXA. Results revealed a total lean body mass increase of +1.8% (+1 kg) in the P0 group and a -1.5% (-1 kg) decrease in the P2 group ($p<.05$) following 12 weeks of resistance training.

Dynamic strength improved in both groups but to a greater extent in the P0 group with differences between groups non-significant. The P0 group improved in more isokinetic strength measurements than the P2 group but not significantly. Cross-sectional area increase was significantly higher ($p<.01$) in the P0 group from pre-training to post-training (7% increase) compared to the P2 group (0% increase). Mean fiber area increased relatively more ($p<.01$) in P0 (+22%) than P2 (-5%). The authors concluded that timing of protein intake following an exercise bout is important for protein synthesis and skeletal muscle hypertrophy in the elderly. This study was one of the first to explain the importance of protein supplement timing combined with resistance training on muscle strength and morphological parameters among the elderly.

In an 2009 trial by Verdijk, elderly males (N = 26) completed 12 weeks of resistance training to measure differences in muscle mass, body composition, and strength when giving 10 grams of a casein versus a placebo (flavor masked water) before and after every training session. Participants were asked to refrain from other dietary intake for 90 minutes prior to the pre-exercise supplement and for at 120 minutes after the post-exercise supplement. Measurements included 1-RM strength, DXA, limb CT scans, and muscle biopsies. Both supplement groups consumed reported diets that were similar in reported energy intake and maintained their protein intake at 1.1 g/kg body weight. Both groups increased strength and revealed muscle fiber hypertrophy, however no group differences were observed in any of the outcome measures.

Andersen et al. (2004) aimed to study the effect of resistance training combined with timed protein supplementation on muscle size and strength. The double-blind,

randomized, matched by strength study included 22 men assigned to a protein experimental group or a carbohydrate control. Exclusion criteria included elite athletes, previous resistance training, and supplement use. Participants took either a protein or carbohydrate isocaloric supplement (25 g each) before and after exercise and once on non-exercise days. Training included 3 exercise sessions per week for 14 weeks and featured various leg exercises such as leg press, knee extension and leg curls. Muscle biopsies were taken from the leg at pre-intervention and post- intervention training to assess muscle fiber size. Vertical jump and isokinetic peak torque were measured to evaluate strength. Four-day food records were kept and analyzed prior to the training period.

The authors reported that hypertrophy was observed in type I and II muscle fibers of participants in the protein supplemented group. Type I and II fibers increased 18% ($p<.01$) and 26% ($p<.001$) respectively from baseline in the protein group. No significant changes occurred from baseline to endpoint in the carbohydrate group muscle fibers. Increases in squat jump (9%) were observed in the protein group from baseline to endpoint ($p<.01$) whereas no significant increase in squat jump performance occurred in the carbohydrate group. Countermovement jump height increased significantly in both supplement groups. Peak torque increased from pre-training to post-training in both groups with nonsignificant changes between groups. The authors concluded that their results revealed minor advantages of protein supplementation over carbohydrate as indicated by improvements in mechanical muscle function and muscle hypertrophy.

In a recent study, Cribb & Hayes (2006) determined the differences between supplementing before and after exercise versus supplementing at different times during the day on muscle hypertrophy, strength development, and body composition following a 10 week resistance training intervention. Twenty-three recreationally trained male bodybuilders were matched by strength and randomized into one of two supplement groups: PRE-POST-exercise consumed their supplement immediately before and after workouts while MOR-EVE consumed their supplement early in the morning and late in the evening at least 5 hours outside of their workout. All participants received supplementation on training days only. The supplement was given at a dose of 1g/kg and contained per 100g: 40g whey protein, 43g glucose, <1g fat, and 7g of creatine monohydrate. Participants were encouraged to maintain their usual dietary intake over the course of the intervention and completed three separate 3-day diet records. All volunteers participated in a structured and supervised resistance training program designed to increase muscle size and strength. DXA was used to determine body composition while muscle biopsies were collected to assess muscle fiber types, cross sectional area, contractile protein content, and metabolite concentrations. No differences over time were observed in either group with regard to dietary intake. The PRE-POST group significantly increased their lean body mass from 69.5 kg to 72.3 kg ($p<.05$), decreased their percent body fat from 13.7 to 12.6 ($p<.05$), and significantly increased their squat and bench press 1-RM. No significant changes in body composition or strength were observed in the MOR-EVE group. In addition, the PRE-POST group experienced significant increases in type IIa and type IIx fiber cross sectional area and had higher

levels of phosphocreatine, total creatine, and glycogen in muscle samples at study endpoint. This study provides further evidence of the benefits of supplementation given before and/or after resistance training on muscle strength, body composition, and muscle hypertrophy.

In contrast to the effects seen in these chronic studies, significant body composition changes have not been replicated in all supplement and resistant training research. Two recent studies that recruited untrained young males examined changes in body composition and strength following 10 weeks of resistance training and post-exercise supplementation and found no significant results between control and experimental groups. Chromiak et al. (2004) examined a 370 kcal whey protein, EAA, creatine, and carbohydrate beverage compared to an isocaloric carbohydrate only beverage immediately following 4 days/week resistance training. Results from hydrodensitometry assessment revealed only a trend toward increasing fat free mass in the supplement group from baseline (+3.4 kg) versus control (+1.5 kg) ($p=.07$). It is possible that the length of the study, decreased number of participants from dropout, the lack of diet control, and the caloric density of the control supplement may have prevented significant findings.

In accordance with these findings, Rankin et al. (2004) examined the effect of post- resistance exercise Gatorade supplementation (5 kcals/ kg) versus isocaloric low-fat chocolate milk on strength and body composition (DXA) in 19 young untrained men. In this trial diet was controlled and training occurred 3 days/week. Following the intervention, all study participants increased muscular strength, decreased percent body

fat, and increased fat free mass. The authors cited a trend of increased fat free soft tissue mass in the milk group versus the Gatorade group (p=.13), and noted that a longer intervention may have produced significant findings.

It is important to note that increases in muscle size and cross-sectional area are not the primary mechanisms associated with strength increases in short resistance training trials lasting less than or equal to 8 weeks. Early strength increases that occur at the beginning of a resistance training program are a result of neural adaptation and increased neural drive and overt changes in muscle hypertrophy are not observed until approximately 8 weeks of a resistance training program and (Gabriel, 2006; Staron, 1994). Therefore, longer training programs (>12 weeks) may be beneficial when desiring to maximize the ability to assess strength augmentation primarily associated with increased muscle hypertrophy.

Table 2 Studies involving protein supplement timing

Study	***Exercise***	***Study Length***	***Diet Intervention***	***Measurements***	***Subject Characteristics Training status***	***Results***
Rasmussen et al. (2000)	45 minute 1 time heavy leg RT bout	2 acute bouts per group	2 groups **1hr/3h post-RT** 6gEAA/ 35g CHO or placebo	Leg kinetics: Phenyalaline infusion, Muscle biopsies	N=6 M/F; 41-43 yo; recreationally active adults without consistent RT	Supplement increased muscle protein anabolism after training; breakdown did not change
Esmarck et al. (2001)	RT 3X/week 3 exercises	12 weeks	2 groups: protein **5 min vs 2hr post-RT**/ 10g prot gel. 100kcals	Food records, DXA CS muscle area Muscle strength	N=13 elderly men; avg 74 yo No recent RT	Immediate supplementation Administration ↑CSA, MFA sig. vs 2hr post-exercise
Levenhagen et al. (2002)	60 min and 60% VO2 on cycle	Repeat time measure X 3	1) Kcal free plac. 2) 8g CHO, 3g lip 3) 10g prot, 8g CHO, 3g lipid **post-exercise**	Protein synthesis with leg kinetics	N=5 males, 5 females; 20-41 yo Healthy adults	Group 3 with only positive response: ↑ leg uptake of EAA and leg protein gain
Miller et al. (2003)	Heavy leg RT	Repeat time measure X 3	1) CHO 5g/kg 2) AA .087g/kg 3) Mix; **given 60 and 120 min post-RT**	Leg kinetics: protein synthesis Blood samples 0-240 minutes post-exercise	N=6 male, 4 female; healthy adults; training status unknown	Combined effect of mix reflects sum of groups 1&2; no effect of second drink on first drink

Table 2 Studies involving protein supplement timing

Study	***Exercise***	***Study Length***	***Diet Intervention***	***Measurements***	***Subject Characteristics Training status***	***Results***
Tipton et al. (2004)	1 bout heavy leg RT 80% max 10 sets of 8 leg ext	Time measure 1X per group	1) placebo 2) casein 20g 3) whey 20g **60 minutes post-RT**	Leg kinetics: blood samples 0-300 minutes post-exercise; leucine	N=23 mixed gender; 23-28 yo Healthy adults No recent RT	Both proteins ↑ muscle net protein balance; different serum [AA] observed
Andersen et al. (2004)	3X/wk RT: 3 leg exercises	14 weeks	Protein vs. cho control (isocaloric) 25g **1 hr Before and immediately after RT**	Peak torque, muscle biopsy, vertical jump, Food records	N=22 healthy males; avg age 23 "recreationally active" No recent RT	Muscle fiber size ↑ in protein group ($p<.01$). Significant ↑ in squat jump from pre-exercise to post-exercise in protein group only
Tipton et al. (2001)	10X8 & 8X8 leg press/ext. @ 80% 1-RM	2 acute trials per subject	Essential 6g EAA/35g CHO solution given immediately **pre/post-exercise**	Leg kinetics with L-phenylalanine/muscle biopsies	N=3males;3=females; avg age 30 "recreationally active"	↑ protein synthesis for pre-drink; ↑ AA delivery vs post-drink ($p<.05$)

Table 2 Studies involving protein supplement timing

Tipton et al. (2003)	8 sets of 8 reps leg extension; 80% 1-RM	2-24 hour acute trials	1 supplement group: 2 X 15g EAA in 350ml; **given before and 1 hr after**	Net balance across leg for 4 amino acids; Fractional synthetic rate	N=7, male and females; avg age 27; recreationally active/ No RT	RT and EAA supplementation over 24hr signif. ↑ net balance vs. rest; response of trial is additive to rest
Elliot et al. (2006)	10 sets of 8 rep leg extension; 80% 1-RM	1 acute RT bout per group	1)237g fat-free milk (FM) 2) 237g whole milk (WM) 3)FF isocal (IM) **Post-RT**	Amino acid balance across the leg	N=24 male and female; avg age 26; no RT X 5 years	Arterial [AA] ↑ in all groups; WM caused greatest uptake of AA
Borsheim et al. (2004)	10 sets of 8 rep leg extension; 80% 1-RM	2 acute cross-over trials	2 isoenergetic groups 1) Whey, AA, CHO 2) CHO alone **Post-RT**	Phenylalanine uptake across leg and muscle biopsies	N=8 healthy male and female; recreationally active; avg age 29	AA uptake higher in protein group (p=.04). Protein balance larger in protein group

IGF-I, IGFBP3, and growth hormone response to resistance training

Insulin like growth factor-I (IGF-I), IGF binding protein-3, and growth hormone response to resistance training have been studied in the context of several different exercise designs. Hormone studies have examined acute and chronic resistance exercise protocols as well as resistance training with various exercise intensities, body mass stimulation, volume, contraction types, endurance training protocols, muscle damage protocols, and overnight responses. Kraemer & Ratamess (2005) explained that the stimulus created from resistance training creates an environment of hormonal change that plays a key role in muscle alteration. These changes can ultimately impact force generation, power production, as well as tissue growth and remodeling. Hormonal responses to acute resistance training occur to a greater extent compared to chronic changes and appear to be more critical to tissue growth and remodeling. Acute increases in serum hormone concentrations increase the probability of their interaction with target tissue cell membranes. This interaction may help promote muscle protein synthesis within the limits of the training program, genetic predisposition, gender, fitness level, and the potential for adaptation. The primary factor for mediating acute hormonal elevation is creating a proper resistance exercise stimulus including elements related to intensity, volume, rest intervals, exercise selection and sequence, repetition velocity, and frequency.

There are several hormones that are affected by resistance training. The hormones with anabolic and catabolic properties that are most relevant to tissue remodeling include: testosterone, growth hormone, cortisol, insulin like growth factor, insulin, and the

catecholamines (Kraemer & Ratamess 2005). When studying these hormones, their adaptation to resistance exercise is usually categorized as acute changes following exercise, chronic changes while resting, chronic changes from long-term acute resistance training, and changes in receptor content (Kraemer & Ratamess 2005). It is also important to note that there are several confounding factors that can influence hormone concentration including nutrition, training experience, gender, age, diurnal variations, and changes in exercise protocol (Kraemer and Ratamess 2005).

IGF-I

Some acute trials examining IGF-I response have suggested essentially no changes when manipulating load intensity (Raastad et al. 2003), comparing age groups (Kraemer et al. 1999), or observing nocturnal response following heavy resistance training in healthy males (Nindl et al. 2001). However, Bamman et al. (2001) observed a 62% increase in IGF mRNA in both males and females following eight sets of squats suggesting an initial alteration in the IGF system in response to acute resistance training. When supplementation of carbohydrate and protein are added in conjunction with training, the IGF response was shown to be consistently higher than in subjects taking placebo (Kraemer et al. 1998). Chronic trials ranging in duration from 10 to 26 weeks and featuring untrained males and females have shown mixed results across different designs. Ballard et al. (2005) reported elevated IGF-I levels in subjects supplemented with protein and participating in both resistance and endurance training over the course of 26 weeks. Borst et al. (2001) examined various resistance training volumes over 25 weeks and suggested that observed IGF-I increases were related to chronic strength gains.

In contrast, Walker et al. (2004) found no changes in IGF-I response to whole body resistance training over the course of 10 weeks without dietary supplementation. In shorter trials, IGF-I concentrations have decreased in response to negative energy balance caused by 7-days of strenuous training and dieting. In the presence of mixed training/diet induced energy deficits (-2,052 kcals), Nemet et al. (2004) found significant reductions in IGF-I, while Nindl et al. (2003) observed similar reductions in IGF-I concentrations in men following severe energy deficits and sleep deprivation during military operational field training.

IGFBP-3

A significant decrease of 20% was observed in IGFBP3 levels in the second half of a 25 week training program in men and women who performed 3 sets of circuit training 3 days per week (Borst et al. 2001). Ballard et al. (2005) did not observe chronic changes in IGFBP3 over the course of 26 weeks of training in participants with similar characteristics who participated in a combined endurance training and resistance training program while receiving dietary supplementation. In contrast Kraemer et al. (1999) found IGFBP3 concentrations increased significantly in young men, but not older men following a 10 week resistance training protocol consisting of exercise 3 days per week. IGFBP3 response to chronic resistance exercise has also been investigated in untrained versus trained men (Rosendal et al. 2002) as well as younger versus older men (Kraemer et al. 1999). Rosendal et al. (2002) found that IGFBP3 concentrations decreased dramatically and IGFBP proteolysis increased significantly in untrained males but remained unchanged in the well trained subjects. All participants in this study were

military volunteers who participated in 2-4 hours of training daily for 11 weeks. The design did not account for nutrition intake or subject nutrient needs. This is important because severe nutrition deficits of energy and protein have been shown to negatively impact the IGF system (Ross 2000; Nindl et al. 2003). In acute examinations, Nindl et al. (2001) studied the nocturnal response to an acute, high volume (50 set) resistance training trial and found that IGFPB3 increased significantly one hour post-exercise but not overnight.

Growth Hormone

Most of the recent literature examining growth hormone in response to resistance training is acute in nature. These trials have studied recreationally trained males and have concluded that the growth hormone response is largely intensity dependent. Williams et al. (2002) has shown that acute high intensity training induced the largest GH response when compared to low and moderate intensities. Both Durand et al. (2003) and Ahtiainen et al. (2003) also reported intensity related growth hormone elevations by observing larger GH response to concentric contractions compared to eccentric contractions and with forced repetitions (with assistance) versus maximum repetitions (to failure). Additionally, Hansen et al. (2001) noted that increasing the percent of body mass stimulated further increased acute GH concentrations when leg training was added to an arm training routine in young men. Dietary supplementation effects on GH concentrations have also been observed. Kraemer et al. (1998) found that protein/carbohydrate supplementation given 2 hours before training and immediately after consistently enhanced the GH response compared to subjects receiving placebo

during a 1-week crossover study. As a result of a chronic trial without supplementation, Kraemer et al. (1999) reported no change in serum GH in young and older men measured resting at weeks 0, 3, 6, and 10 over the course of 10 weeks of resistance training program.

It is important to note that most of the recent research examining GH, IGF-I, and IGFBP3 response to resistance training studies the male gender or uses a mixed gender sample and does not test for gender effects. The hormonal response seen in this research may not clearly define the true response in untrained women. Both Kraemer et al. (1993) and Taylor et al. (2000) observed spikes in GH concentration immediately following resistance exercise in female subjects. Kraemer et al. (1993) studied nine recreationally trained females participating in 6 different resistance exercise protocols and concluded that exercise sessions that are more glycolytic produce higher acute GH concentrations. Taylor et al. (2000) examined the acute GH response to a whole body resistance training session (7 exercises at 3 X 10-RM) with one minute rest sessions in both untrained and trained females. Growth hormone elevations were seen immediately following exercise in both groups, but in the trained group, the baseline GH concentration was surprisingly lower than in the untrained. The authors concluded that GH response in women varies with training status (Taylor et al. 2000). Hakkinen et al. (2000) conducted a 6-month trial to examine the effects of heavy resistance training on basal and acute GH levels in untrained men and women all older than 36 years of age. Acute elevation of GH was seen immediately following exercise in all groups but basal levels essentially remained

unchanged. The lack of chronic GH response to resistance training in untrained women has also been observed following 24 weeks of both circuit-type and periodized training.

The most relevant work related to resistant training and IGF-I, IGFBP-3, and GH combined with supplementation are presented in greater detail below. This is followed by a chart summarizing various trials to date that have investigated hormonal response to resistance training.

Walker et al. (2004) measured changes in the growth factors IGF-1 and myostatin along with increases in muscle strength and size as a response to resistance exercise training. Seventeen males participated in the study and completed training in one of two exercise groups consisting of either elbow flexor training (n=6) or whole body training (n=11). The elbow flexor group completed 3 total exercises while the whole body group completed nine including the elbow flexor routine. Participants in both groups trained twice a week for 10 weeks. Muscular strength and endurance along with MRI to assess cross-sectional muscle area were measured at baseline and following the 10-week intervention. Blood samples to assess plasma IGF-1 and myostatin were taken pre-training and post-training. Strength and muscle cross-sectional area increased significantly in both groups ($p<.001$), but did not demonstrate significant group differences. No overall change within or between groups was evident for plasma IGF-1. Mean percent change for myostatin revealed approximately a 25% decrease (elbow flexor) and a 15% decrease (whole body) without significant differences between groups. The authors concluded that the amount of muscle mass exercised had no effect on resting IGF-1 and myostatin, therefore, local muscle assessment of these hormones may provide

more useful information. Additionally, the usefulness of IGF-1 as a co-indicator of muscle response may be discounted with this type of resistance training protocol. Factors that need to be controlled in a resistance training program include frequency of training, duration of protocol, and rest intervals.

Ballard et al. (2005) examined the effect of protein supplementation during 6 months of strength and conditioning on IGF-I and markers of bone turnover. Twenty-eight males and 23 females participated in the study. Volunteers were separated into two separate isocaloric supplemented groups and received their supplementation twice a day. The carbohydrate group consumed 70 g CHO while the protein group consumed 42 g protein/ 21g CHO/1.5 g fat. The exercise training protocol included endurance exercise and resistance exercise on alternate days for 5 sessions per week. Proper exercise intensities were maintained by regularly checking 1-RM for strength training and using digital monitors to maintain proper HR range during endurance sessions. Dietary intake was monitored during the study with three-day food records. Study measurements included alkaline phosphatase (BAP), urinary NTx, plasma IGF-1, and IGF-1 binding proteins and were collected at baseline, 3 months, and 6 months. Energy intake increased similarly among groups as the study protocol progressed. Plasma IGF-I increased significantly ($p=.01$) in the protein supplemented group from baseline to 6 months. IGFBP-3 measurements never reached significance over time but was elevated in the protein group at 3-months and returned to approximate baseline levels at the 6-month measurement. NTx, a marker of bone turnover, was consistently higher in men and was the only outcome that revealed gender differences. BAP and NTx levels were

consistently higher in the protein group over time. The authors concluded that protein supplementation during 6-month strength and conditioning program increased IGF-I serum concentrations with both exercise intensity and energy intake similar in both test groups.

Williams et al. (2002) studied acute hormone responses to various resistance training protocols with and without post-exercise carbohydrate-protein supplementation. Seven moderately resistance trained men participated in a randomized, balanced, double-blind, crossover design. The study protocol included six different test conditions classified by exercise intensity and supplement received: LOW, MOD, or HIGH intensity each with supplement or placebo. Both the LOW and MOD exercise groups utilized the leg extension exercise, but the MOD group added intensity by increasing repetition volume. The HIGH intensity group performed 3 sets of 8 different exercises focusing on all major muscle groups. Participants received either a carbohydrate-protein supplement dosed by weight (1g/kg glucose, 0.25g/kg wheat hydrolysate, and 0.125g/kg leucine & phenylalanine) or a similar tasting placebo with minimal energy content. Washout periods between treatment groups were 1-2 weeks. Blood samples were taken at various time points, including rest (prior to exercise) and at time 0, 15, 30, 60, and 120 minutes after exercise on six separate lab days. Plasma glucose, lactate, cortisol, insulin, and growth hormone were measured. Changes in growth hormone were only seen in the HIGH group and occurred immediately post-exercise (time 0) while peaking at 4.5 micrograms/liter at 15 minutes post-exercise. Growth hormone remained significantly higher ($p<.05$) than LOW and MOD up to 30 minutes post-exercise. Supplementation

had no effect on growth hormone profiles. The authors concluded that interactions between resistance exercise volume and nutritional supplementation on post-exercise growth hormone concentration did not exist. Furthermore, the authors suggested that post-exercise carbohydrate/protein supplementation may not influence growth hormone response independently, but may be useful in stimulating muscle protein synthesis by providing an exogenous amino acid source and promoting an enhanced insulin response. It is important to note that the usual and protocol diets of participants were not assessed in this study.

Table 3 Resistance training induced hormonal changes

Study	***Hormones***	***How hormones measured***	***Exercise protocol***	***Subject/Study Characteristics Training status***	***Other study dep. variables & length***	***Diet: Controlled? Supplemented?***	***Results***
Borst (2001)	IGF/ IGFBPs 1&3	3X during study length; time course with strength increases	3 groups 1)single set RT 2) multiple set RT 3) no exercise control 3d/wk-7 exercises/8-12 reps	N=31 mixed gender sedentary	Strength gains with RT/ 25 weeks	No	Suggests in part ↑ IGF-I mediates ↑ strength; ↑IGF-I for first ½ of protocol in groups 1 and 2
Walker (2004)	IGF-1, Myostatin	Pre/post-training	2 groups: elbow flexor (3 ex) vs whole body RT(9ex). Trained 2X/wk	N=17 males untrained pre-training versus post-training	Strength, endurance and muscle size; 10 wk protocol	No	No change in IGF-I Question frequency, intensity, and duration of protocol
Williams (2002)	Cortisol Growth hormone Insulin	Acute response/ blood measures over time	Low, mod, high intensity RT training groups; "normal" training b/t sessions	N=7 moderate RT trained males Crossover design;	Lactate, serum glucose; Repeated crossover design	Post-training CHO(1g/kg)/prot (.25g/kg) beverage or placebo	GH ↑ in high intensity group; Supplementation = no effect
Ballard et al. (2005)	IGF, IGFBP3	Fasting at baseline, 3mos, and 6mos	Alternating RT (70%1-RM) and ET(70-80%mhr) 5 days/week; intensities maintained	N=28 males, N=23 females untrained	Bone t/o markers; Cardiovascular assessment; 6 month protocol	2 supplement groups: 70g CHO vs. 42g protein/ 21g CHO/1.5g fat (2X/day) ; monitored food records	Plasma IGF-I ↑ in protein group (p=.01); No change in IGFBP3 No hormonal gender differences

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Study	***Hormones***	***How hormones measured***	***Exercise protocol***	***Subject/Study Characteristics Training status***	***Other study dep. variables & length***	***Diet: Controlled? Supplemented?***	***Results***
Durand et al. (2003)	growth hormone, testosterone and free testosterone	Blood samples post-exercise & 15min post-exercise	3 different acute trials Preliminary, concentric, eccentric; 4 exercise 4X12 (80%1-rm)	N=10 young males counterbalance design recreationally trained	Lactate	No control/ supplement; nutrition screening for inclusion	Concentric ↑ [GH] more than Eccentric ? if ↑ more a fxn of intensity instead of contraction
Bamman et al. (2001)	IGF-I, IGF-I & IGFBP4 mRNA	Pre-exercise and serially across 48 hours after RT	8 sets of 8 squats with Concentric Vs. Eccentric Load	N=7males; n= 3 females healthy ambiguous base training status	Androgen receptor mRNA, testosterone, CK, isometric MVC, soreness	Controlled isocaloric diet 8 hours post-training during blood draws	Single loading bouts alter IGF-I system; IGF-I mRNA ↑ 62%/ BP ↓57% ($p<.05$) with ECC
Ahtiain et al. (2003)	testosterone cortisol, GH	Basal levels & pre-ex., during, and up to 72 hr post-RT	Max rep and Forced rep RT protocol/ leg press, squat, knee ext	N=16 males; physically active Recreational RT	Max isometric force/ recovery force/anthropo -metrics	NO	GH ↑ ($p<.01$) with FR compared to MR
Raastad et al. (2003)	Cortisol, growth hormone, IGF	Both pre/post-exercise protocol, following a test trial	Exp. group: moderate-intensity high-volume RT; Heavy=multi joint/daily; control: RT2X/wk	Resistance trained males n=17; random assignment to exp. versus control groups	neuromuscular fatigue, recovery; 2 weeks	Meals controlled during trials	Only essential change occurred with cortisol

Table 3 Resistance training induced hormonal changes

Study	***Hormones***	***How hormones measured***	***Exercise protocol***	***Subject/Study Characteristics Training status***	***Other study dep. variables & length***	***Diet: Controlled? Supplemented?***	***Results***
Goto, Sato, and Takamat (2003)	GH	Pre-exercise and multiple time periods post-exercise	4 different acute leg extensor protocols; strength type +various %1RM low intensity sets	N=8 recreational resistance trained fasting males	lactate	Subjects asked to maintain diet	C50 type signif. ↑ GH ($p<.05$) vs. S-type and C90 type; High intens. /low volume produce little GH change
Nindl et al. (2001)	Overnight [GH] Compared 3 different assay techniques	overnight series blood sampling for repeated measures	acute heavy resistance training bout; 50 sets of multi-joint RT exercises beginning at 1500	N=10 physically fit men Served as own control	physical characteristics, aerobic fitness, and muscular strength	Metabolic unit; 3-day diet recalls before each trial	Max [hGH] and mean pulse amplitude lower in exercise groups
Nindl et al. (2001)	Evaluated nocturnal IGF system to acute heavy RT protocol	overnight series blood sampling for repeated measures	acute heavy resistance training bout; 50 sets of multi-joint RT exercises beginning at 1500	N=10 physically fit men Served as own control		Metabolic unit; 3-day diet recalls before each trial	Only significant difference: IGFBP3 ↑ in exercise group 1 hr post-exercise
Rubin et al. (2005)	GHBP	Repeated time points from pre-trial to 60 minutes post-trial	6 sets of 10 parallel squats	Cross-sectional N=9 males resistance trained N=10 untrained males	Acute training; Body composition/ BMD & RPE	24 hr recall X 3	↑IGF-I,GHBP,iGH seen across groups; iGH & IGF-I signific. ↑ in trained group

Gender differences in lean body mass as a result of resistance training

Chronic resistance training is known to optimize the development of lean body mass across gender. Muscle hypertrophy represents a primary gender difference as a result of training. Kraemer et al. (1991) suggests that men experience greater absolute hypertrophy mainly due to a greater anabolic hormone response. Testosterone is one of these anabolic hormones that have been shown to be 20-30 times higher in males than females on average over a continuum (Kraemer et al. 1991). Gender differences in bone and muscle cross sectional area have also been examined after resistance training. Cureton et al. (1988) observed that a 16 week resistance training program resulted in similar percent change in bone and muscle in both men and women from training by computed axial tomography. Only the absolute change in bone and muscle was greater in men. This research suggests that lean body mass, specifically muscle, is generally greater in males mainly because of larger initial muscle mass and anabolic hormone response to training.

Body composition and weight loss changes from resistance training

In a meta-analysis of 53 studies (Ballor & Keesy, 1991) resistance training was found to have a noteworthy effect on the preservation of fat free mass in both males and females participating in weight loss programs. Changes in body composition was also looked at by Broeder et al. (1997) who examined 12 weeks of high intensity resistance exercise versus endurance exercise in non-dieting males. Changes in body composition were measured by bio-electrical impedance and underwater weighing. The results of this trial show that participants undergoing endurance training alone decreased fat weight

without producing changes in fat free mass. In contrast, participants who underwent resistance exercise alone increased fat free mass while decreasing fat mass. An individual's inherent body build has also been shown to have an impact on body composition changes resulting from resistance training. Van Etten et al. (1994) reported that when matched for muscle mass, males with greater muscle mass experienced significantly greater increase in fat free mass from 12 weeks of resistance training (2 exercise sessions per week) compared to slender male counterparts. However, both groups of males experienced similar increases in strength and decreases in fat mass.

Ballor et al. (1988) showed that resistance training combined with caloric restriction enhanced lean body mass maintenance and weight loss in obese women. Weight loss in volunteers who participated in caloric restriction alone (-4.47 kg) and resistance training with caloric restriction (-3.89 kg) lost significantly more weight than subjects who only resistance trained (-0.45 kg) or controls (-0.38 kg) after 8 weeks. Furthermore, lean body weight only increased in the women who resistance trained (+1.07 kg) and the women who trained and restricted calories (+0.43). One of the reasons for resistance training induced changes in weight and body composition may be due to changes in resting metabolic rate (RMR). Dolezal and Pottieger (1998) compared concurrent endurance training and resistance training with resistance training and endurance training alone on thirty physically active males. Results indicated that only males participating in resistance training experienced significant rise in resting metabolic rate. In a 14 week trial examining the effect of resistance training during diet induced weight loss on body composition, Nakata et al 2008 studied 42 overweight Japanese

women. Participants were randomized to a diet only group (D; n = 17 completers) or a diet plus resistance training (DR; n = 18 completers). The diet protocol for both groups consisted of a goal energy intake of 1200 kcal per day with supplemental food products to maintain a calcium intake of > 800 mg per day. The primary outcome of this study was bone mineral density (BMD) and the authors found no significant differences in DXA BMD or BMD related markers. From a soft tissue standpoint in this protocol, fat mass reductions were greater in the DR group than the D group (-6.9 kg vs. 4.5 kg; $p < 0.019$) and lean mass losses were significant in both groups by time.

In a longitudinal, clinical, weight loss trial, Hunter et al. 2008 studied racial differences in body composition, strength, and resting energy expenditure following concurrent resistance training and a weight loss diet. Overweight African American (AA) or European American (EA) were randomized to aerobic (AT), resistance (RT), or no training (NT) groups and underwent weight loss to a normal BMI. AA women lost less fat-free mass than there EA counterparts ($p < .05$). RT women, independent of race, were the only to maintain FFM as a result of weight loss ($p <.05$). In addition, the RT group was able to maintain resting energy expenditure from baseline compared to NT and AT ($p < .05$), while also maintaining strength compared to AT ($p < .05$). Based on the results to this trial, RT appears to be essential to maintaining FFM, resting energy expenditure, and strength while undergoing a diet induced weight loss.

Summary

Epidemiological and observational research supports the role of dairy in promoting weight reduction and fat loss. In addition, much of the clinical data support the role or dairy as a catalyst for promoting weight and fat loss, while other research suggests that the effects of dairy is marginal and inconsistent. The mechanism for dairy in weight regulation and body composition is complex and not fully understood. It is thought that calcium, BCAAs, and ACE inhibitors contribute to dairy bioactivity. It is clear that larger clinical trials are needed to better assess the role of dairy in body weight regulation, body composition, and fat distribution. Intervention trials that feature increased dairy consumption and mild caloric restriction have never examined the combined effect of resistance exercise on body composition outcomes and should be the focus of future research.

Exercise is a proven strategy for improving lean body mass. This effect is pronounced as a result of chronic resistance training. Weight bearing exercise alone is known to improve strength, lean body mass, resting metabolic rate, and assist in chronic weight management. In addition, evidence is mounting that timed protein/carbohydrate supplementation occurring before and after each training bout can assist in muscle amino acid uptake by increasing amino acid availability and by promoting a favorable insulin response. Timed supplementation may help maximize lean body mass accretion during chronic resistance training. To date, there are no chronic studies examining the combined effect of timed supplementation and resistance training on body composition.

Resistance exercise clearly influences the acute hormonal response of GH and IGF. However, chronic changes in growth hormone are generally absent with training while IGF changes are conflicting. Studies that examine nutrition supplementation combined with resistance training on IGF/GH response are limited in number and report inconsistent results. It is difficult to generalize the results of these studies based on differences observed in participant characteristics and training history, supplement schemes and exercise protocols. Untrained women appear to have large acute hormonal responses to exercise. More studies are needed to elucidate hormonal response in sedentary women who partake in chronic resistance training and protein supplemented trials designed to improve lean body mass. Hormonal responses in a well controlled study may help to explain observed changes in strength and body composition.

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CHAPTER III

THE EFFECTS OF A HIGH DAIRY DIET AND CHRONIC RESISTANCE EXERCISE ON IMPROVING BODY COMPOSITION IN OVERWEIGHT SEDENTARY WOMEN

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Abstract

Previous reports suggest that high dairy calcium diets help augment total and regional fat loss in obese women. The objective of this study was to examine the calcium and fat loss hypothesis in overweight women with chronic low calcium diets who participated in a resistance training program with calorie restriction. Participants (age = 36.6 ± 4.7; African American 57.7%, White 30.8%, 11.5% other) with a BMI of 29.1 ± 2.2 kg/m² were randomized to low calcium (LC) (≤ 500 mg; n=13) or high calcium (HC) (≥1200 mg; n=13). All participants received reduced calorie (250 kcal deficit) diets. Six dietary recalls were obtained by a multi-pass approach provided by Nutrition Data System software. Body composition was measured by dual energy x-ray absorptiometry, waist circumference, and sagittal diameter. Participants completed 16 weeks of whole body resistance training three times per week. Mean weight loss in the total sample trended toward significance (1.9 kg; $p = 0.06$) and corresponded to significant caloric reduction from baseline ($p = 0.001$). The prescribed mean calcium intake was achieved for each study group (LC = 469.0 ± 148.3 and HC = 1297.0 ± 181.5 mg) with no significant changes in protein intake over time (LC = 0.92 and HC = 1.02 g/kg, $p = 0.21$). Fat mass index (LC = 12.3 to 11.0 and HC = 13.0 to12.2 fat kg/m²), trunk fat (LC = 1.74 to 1.54 and HC = 1.68 to 1.55 kg), waist circumference (LC = 88.4 to 85.0 and HC 84.6 to 82.3 cm), and sagittal diameter (LC = 27.1 to 25.8 and HC = 25.6 to 24.4 cm) all significantly decreased over time ($p \leq 0.05$) with no group differences ($p \geq 0.37$). These data suggest that high dairy calcium diets offer no added benefit in reducing fat indices when combined with resistance training and caloric restriction.

Introduction

The National Center for Health Statistics reports 61.4% of U.S. women ages 20 to 74 are overweight while 34% are obese.[1] Obesity and overweight are related to more than 30 serious medical conditions including type II diabetes, coronary heart disease, hypertension, and osteoarthritis. Given the association between fat oxidation and dairy intake observed in recent trials, [2-7] increasing low fat dairy intake may help overweight women improve their body composition and help prevent further weight gain over time. In support of these findings, observational studies suggest an inverse relationship between dietary calcium intake and body weight and decreased indices of adiposity.[8-9] However, not all studies support this relationship. In a recent observational trial,[10] Vergnaud and colleagues studied 6-year changes in body weight and waist circumference changes in 1022 women and found no significant relationship between dairy product consumption and weight decrease in overweight and normal weight women.

In clinical trials, the effect of dairy treatment on loss of fat mass remains a topic of debate. Trials by Zemel et al. have shown a significant reduction in fat mass with dairy treatment and concomitant energy restriction, [11-13] while other groups were unable to report similar findings. [14-16] Clinical trials designed to re-examine the effect of dairy and calcium on fat mass changes continue to be equivocal.

In addition to playing a role in fat mass reduction, optimal dairy consumption may promote weight loss maintenance. In a 2007 trial, [17] obese women (N=103) were followed for eighteen months after completing an eight week weight loss trial (mean loss = 9.66 kg). The authors found that calcium and energy intake were correlated and that

there was a significant inverse relationship between calcium intake and weight gain when energy intake was controlled. Conversely, higher energy intake significantly predicted weight gain when calcium intake was controlled. The authors concluded that dietary calcium may slow the progression of gaining weight by reducing the effect of greater energy intake. [17] In a similar trial, [18] participants receiving recommended intakes of dairy (> 3 servings of dairy per day) consistently consumed more energy than low dairy counterparts during the weight maintenance phase of the study. After observing significant group differences in respiratory quotient, the authors concluded that consuming recommended daily amounts of dairy may allow for higher fat oxidation rates that might permit greater energy intake without weight gain.

Exercise interventions designed to examine the dairy and body composition hypothesis are limited in number. A recent 12-week weight loss trial incorporating resistance training and aerobics compared various forms of calcium and skim milk on the magnitude of weight and fat loss.[18] Despite significant weight and fat losses over time, no group differences were observed. This may be partially explained by not controlling for usual calcium intake and the relatively high calcium intake of the placebo group compared to trials that show a calcium effect. [11-13] As a result of an eight-week trial, women who consumed a high dairy diet consisting of three servings of yogurt per day including one serving of yogurt immediately following training sessions were able to significantly decrease percent body fat over time. [19] Similar results by time were seen in other study groups that received isocaloric, non-dairy, post-exercise supplements and

maintained on low calcium diets. However, the yogurt group had a significantly higher calorie intake and similar protein intake compared to other treatment groups.

To date, no clinical trials have been designed to examine the dairy calcium and weight or fat hypothesis combined with structured, 16 weeks of resistance training in an overweight, premenopausal, female sample. It is conceivable that any chronic exercise may confound a benefit of dairy on fat loss and weight maintenance. However, it can be argued that the potential for synergistic benefits of combining dairy and exercise to promote healthful body composition change is of greater interest than focusing on the ability to precisely measure the magnitude of dairy alone on promoting these changes. Furthermore, because of the highly variable outcomes seen in several trials, the role of dairy in weight loss and body composition needs further examination with unique designs.

It is clear that longer clinical trials are needed to better assess the role of dairy in body weight regulation, body composition, and fat distribution. Intervention trials designed to examine increased dairy consumption and mild caloric restriction in women has never been examined in conjunction with 16 weeks of exclusive resistance training. Moreover, research gaps can be narrowed by interventions designed to discover the ideal integration of nutrition and exercise strategies into a successful intervention program that is both practical and uniquely devised for women at risk for further weight gain. It is worth examining the combined effect of controlled physical activity in the form of chronic resistance training and high intake of dairy calcium on maximizing fat loss and body composition changes in overweight sedentary women. Therefore, the primary aim

of this study is to evaluate the effect of a reduced calorie diet high in calcium to promote fat mass reduction and/or prevent further weight gain in overweight women undergoing 16 weeks of resistance training.

Methods

Participants

Participants were recruited using advertisements distributed across the University of North Carolina at Greensboro (UNCG) campus and in the nearby community (see Appendix A). Advertisements clearly stated the purpose of the study, inclusion and exclusion criteria, and benefits of study participation. Potential volunteers were instructed to contact the lead investigator to provide height and weight information for body mass index (BMI) assessment. If the interested volunteer met BMI requirements (BMI 25-30 kg/m^2), a follow up interview was scheduled to assess final eligibility. The follow up interview was guided by two questionnaires (see Appendix B-C). The first questionnaire collected information on items such as exercise, nutrition, and medical history. The second questionnaire was a validated screening form designed to assess pre-exercise health status. [20] Two hundred and forty-two volunteers contacted the lead investigator to take part in the initial BMI screening process. If an overweight BMI was determined, an in depth phone interview was scheduled to determine final eligibility. To meet inclusion criteria, interested volunteers must have had a usual calcium intake of less than or equal to 700 mg/day, 29-45 years of age, deemed appropriate for resistance exercise, and no resistance training in the previous 3 months. The premise for selecting this age group and BMI range was twofold. First, the incidence of obesity increases with age and women of

this age group are at a higher risk for weight gain because of career and family related responsibilities leading to decreased physical activity. Additionally, women of this age group who were already overweight may benefit the most from an intervention designed to prevent further weight gain and therefore, reduce their chances of developing obesity related co-morbidities later in life. Specific exclusion criteria included pregnancy or lactation, calcium supplementation, usual dairy intake of greater than one serving per day, reported aversion to dairy products, previous history of orthopedic injury, GI disease, endocrine disorders, or any other medical condition that could compromise the safety of participation or confound study results. Interested volunteers who were on any medications that could confound study results were also excluded, including: steroidal drugs, diuretics, calcium channel blockers, insulin or anti-diabetic agents, synthetic thyroid hormones, and over the counter weight loss supplements. Oral contraceptive use was recorded but not considered as criteria for study exclusion. During the screening process, two hundred and one volunteers were deemed ineligible based on personal choice or by the established exclusion criteria: age (n = 23), body mass index > 30 kg/m^2 (n = 82), high calcium intake of greater than 700 mg/day (n = 20), under or normal weight (n = 47), scheduling conflicts and personal reasons (n = 19), medical history (n= 10), and actively engaged in an exercise program (n = 5). Thirty-five participants met all eligibility requirements and were invited to the laboratory for baseline measures.

The study was approved by the UNCG Institutional Review Board and all eligible participants gave informed written consent prior to engaging in baseline measures (see

Appendix D). We certify that all institutional and governmental regulations concerning the use of human volunteers were followed during this research.

Study design

This study was a randomized clinical trial. After baseline measurements of diet, muscular strength, weight, and body composition, participants were randomized to either a low calcium diet (≤ 500 mg/day) (LOW) or a high calcium diet (≥ 1200 mg/day) (HIGH) group (**Figure 1**). Random numbers that corresponded to each of the study groups were generated by statistical software and individually placed in sealed envelopes for group assignment. Participants assigned to LOW were asked to maintain their typical low calcium intake while HIGH participants were instructed to increase their calcium intake. Measurements of diet, strength, and body composition were reassessed at study midpoint and endpoint (see Appendix E). The order of events during measurement days were as follows: A) urine sample (to rule out pregnancy), height (baseline only), weight, waist circumference and sagittal diameter measurements, 1-RM strength assessment, and Dual Energy X-Ray Absorptiometry (DXA) scanning (baseline and endpoint only). All volunteers participated in 3 days per week whole body resistance training for 16 weeks and were instructed to follow a nutritious 250 kcal deficit diet 7 days per week for the 16 week intervention. All participants were asked to refrain from participating in additional exercise programs or using dietary supplements throughout the 16 week intervention. The primary goals of this intervention were to reduce fat mass while maximizing the conservation of lean body mass. Therefore, a modest weight loss of approximately 0.25 kg/week was our goal and was attempted with a prescribed daily energy deficit of 250

kcal. Participant weight was documented weekly and used as a tool to assess diet compliance (see Appendix F). In addition to monitoring body weight changes, participants met with the study dietitian three times per week prior to the exercise sessions to discuss compliance and address diet related questions.

Diet intervention

After randomization to either low (≤ 500 mg/day) or high (≥ 1200 mg/day) calcium diets, participants received individualized counseling from a registered dietitian (RD) and were instructed on the use of an exchange system diet to guide prescriptions for energy and daily calcium intake. The prescribed diet was based on the American Diabetes Association (ADA) exchange system [21] and was constructed to control for daily protein consumption (~15% of total calories). The exchange system is a standardized food grouping system that is designed to be easily understood and practiced by followers. This system effectively teaches appropriate serving sizes to control energy and macronutrient intake while also allowing participant autonomy with meal planning. Diets were individualized and designed by the study RD during initial counseling sessions to promote a modest energy reduction (-250 kcals) from baseline energy needs. The primary method for accomplishing this mild deficit was encouraging participants to reduce their sugar and fat intake while attempting to maintain nutritious food variety and appeal (see Appendix G).

Participants in the high calcium diet group received a high calcium food list and were given examples on how to incorporate these foods into their daily exchange plan to maintain their daily dietary intake goal of ≥ 1200 mg (see Appendix G). The primary

strategy for increasing calcium intake was through encouraging the consumption of greater than or equal to three servings of dairy foods per day. Participants in the low calcium group were instructed not to consume any dairy products, avoid any food with greater that 15% of daily calcium value per serving, and taught to avoid naturally occurring non-dairy calcium sources. Participants in both groups received complimentary vitamin D supplements (100% RDA) to take daily in an effort to prevent insufficient dietary intake. In addition, since folic acid is known to prevent neural tube defects and that participants were asked to refrain from personal supplement use, we provided folic acid supplements (100% RDA) to take daily.

Compliance with diet was monitored and maintained by assessing weekly weights, midpoint dietary recall assessment, and providing the opportunity for weekly question and answer sessions designed to address diet adherence. The caloric intake totals from two random baseline 24-hour food recalls were averaged with calculated energy needs for each participant to determine a baseline calorie need estimate. Energy needs for weight maintenance were calculated using the Food and Nutrition Board's equation for determining energy needs in overweight and obese adult women. [22] Adjustment of total energy expenditure (TEE) for physical activity levels (PAL) were accomplished by multiplying the TEE by the appropriate PAL coefficient (sedentary = 1.0 or low active = 1.16). Initial calorie need estimates were determined by averaging initial energy calculations (TEE X PAL coefficient) with the baseline dietary recall information. The initial intervention calorie prescription was determined by subtracting 250 kcal to promote a 0.25 kg weight loss per week. During the course of the 16-week trial, if weight

loss was not progressing as planned, the study dietitian immediately assessed diet compliance and subsequently decided if additional food exchanges needed to be subtracted from the diet plan to create a greater calorie deficit.

Exercise intervention

Whole body resistance training took place three times per week for the entire 16 week study protocol. Participants trained on Monday-Wednesday- Friday or Tuesday-Thursday-Saturday based on their convenience. The exercises used and their progression were as follows: dumbbell chops (for total body warm-up and core stimulation), followed by dumbbell squats, dumbbell bench press, dumbbell rows, and dumbbell dead-lift (see Appendix H). Participants completed all training sessions in the Human Performance Lab at UNCG under the close supervision of trained research personnel. All participants began the training program by completing a 2-week familiarization period followed by 14 weeks of training progression. During the familiarization period, participants completed all exercises with 2 sets of 10 repetitions at 60-70% of their initial 1-RM. In order to provide additional familiarization with the squat exercise, participants performed the exercise (weeks 1-2) using only their body weight with an exercise ball placed against the wall prior to advancing to free weight dumbbell resistance (weeks 3-16). Training advanced to 3 sets per exercise at week three with a goal repetition range of eight to twelve. Rest periods between sets were timed and enforced at 60 seconds. Participant progression followed the classic linear model of periodization as strength improved between 1-RM measuring points. [20] Participants gradually progressed to training at 80-100% of their baseline 1-RM while maintaining a goal repetition range of 8-12. Training

load estimates, based on percent 1-RM, were readjusted at protocol midpoint (week 8) when strength was reassessed with an absolute 1-RM. In order to assist in maintaining intensity and appropriate progression between strength measurements (baseline, midpoint, and endpoint) load adjustments were also based on each participant's ability to stay within the intended goal of eight to twelve repetitions.

Clinical measurements

Height was measured without shoes on a stadiometer (Accustat Genentech) at week 0. Weekly body weight was measured by a stationary balance beam scale in exercise clothing without shoes. Changes in total and regional body composition (fat and lean mass from soft tissue) from study baseline to endpoint were assessed by Dual Energy X-Ray Absorptiometry (DXA) (Lunar-Prodigy Advance Plus). Primary outcomes measured with DXA included total body mass (kg), total body percent fat, fat mass (kg), fat-free mass (including bone mineral content in kg), total lean (minus bone mineral content in kg), and trunk lean/fat (g). Fat mass index [FMI = fat mass kg ÷ height (m^2)] and fat free mass index [FFMI = fat free mass kg ÷ height (m^2)] were derived from DXA fat mass and fat-free mass respectively. FMI and FFMI allow for a 2-compartment analysis of body composition changes and are better suited at assessing absolute changes than evaluating percent fat or percent lean. [23] In addition to DXA analysis, waist circumferences (Gulick II tape measure) and sagittal diameter (Rosscraft Campbell Caliper 20) were measured to assess changes in central adiposity over time. Waist circumferences were measured at the narrowest part of the waist per American College of Sports Medicine guidelines [20] at all three study time points.

Strength assessment

Strength was assessed with one repetition max testing (1-RM) at baseline, midpoint and at the end of 16 weeks. [24] Participants began with a warm up of 5-10 repetitions at 40-60% of the participant's perceived capacity for one lift. After a short rest period of 2 minutes, 3-5 repetitions were completed at 60-80% of the participant's perceived capacity for the same lift. Finally, successive 1-RM attempts were performed until failure with the goal of determining the true 1-RM within 3-5 trials. Loads were increased by 2-5 kg for each trial and participants were allowed to rest 3-5 minutes between attempts. Verbal encouragement was given at each attempt to maximize performance.

Diet

Diet was assessed over the phone using the Nutrition Data System for Research (NDS) nutrition software system. [25] This system utilizes the multiple pass recall method to help improve the validity of dietary data. Two random dietary recalls within the same week occurred prior to protocol start, at midpoint, and at study endpoint.

Statistics

Data were analyzed with SPSS (version 15.0). Differences between groups in baseline characteristics were determined with Student's t-test or Chi-Square analysis. Differences in body composition, weight, anthropometrics, and strength between experimental groups over time and by group were determined by repeated measures analyses (RMANOVA). Race was added as a covariate in analysis since differences in race distribution occurred between calcium groups.

Results

After randomization, six participants withdrew from the study prior to the 16 week protocol commencement due to pregnancy (high calcium, n = 1), scheduling conflicts (high calcium, n = 1), and personal reasons (n = 2, low calcium; n = 2, high calcium). A total of 29 participants are scheduled to complete the 16 week intervention. The data presented are preliminary and represent a sample size of n = 26. There were no significant differences in baseline age, weight, height, or BMI (**Table 1**). Calorie intake (p=0.50), protein intake per kilogram body weight (p=0.3), and calcium intake (p=0.77) were not significantly different at baseline. Measures of strength (1-RM) were similar between groups at baseline. A significant difference in race distribution was observed between groups when race groups were dichotomized as Black (n=5 low calcium; n=10 high calcium) or Other (White, Asian and Latina) (n=8 low calcium; n=3 high calcium).

Weight loss and body composition

Changes in weight and body composition by group are outlined in **Table 2**. Mean weight loss in the total sample trended toward significance over time (1.9 kg; p = 0.06) but not between groups. Total mean weight loss per group over the 16 week protocol was -2.4 ± 4.6 kg (LC) and -1.3 ± 2.5 kg (HC) and represented a mean 2.6 percent weight loss for the total sample. Waist circumference decreased over time in both groups and when race was added as a covariate, LC lost significantly more (-3.4 ± 5.5 cm) than HC (-2.3 ± 2.2 cm) ($p \leq 0.01$) (**Figure 3)**. When controlling for race, mean percent body fat change and fat mass index were significantly different between groups LC (-3.59%/-1.35 kg/m^2) versus HC (-1.89%/-0.75 kg/m^2) ($p \leq 0.05$)(**Figure 2**). Mean sagittal diameter reduction

followed the waist reduction time trend (LC = -1.4 ± 1.4 cm versus HC = -1.2 ± 0.8 cm) ($p = 0.04$) but without group differences. Large reductions in fat mass (LC = -3.5 ± 4.2 kg versus HC = - 2.0 ± 2.2 kg) and trunk fat (LC = 1.98 ± 2.5 kg versus HC = 1.32 ± 1.5 kg) were observed over time ($p \leq 0.05$) but not by calcium group. Intervention calcium intake did not correlate with changes in trunk fat ($r = 0.24$; $p = 0.25$) or fat mass ($r = 0.28$; $p = 0.17$). Females with a waist circumference greater than 88 cm at baseline ($n = 10$), and considered at risk per ACSM guidelines,[20] lost 4.91 cm in waist circumference compared to 1.54 cm in females with lower baseline measures ($n = 16$) ($p = 0.09$). Total lean tissue increased in both groups over time (LC = 1.23 ± 0.8 kg versus HC = 0.76 ± 1.3 kg) ($p \leq 0.05$) but without group differences.

Dietary composition

Dietary intake characteristics by group over the course of the study trial are outlined in **Table 3**. The high calcium group achieved their goal calcium intake as evidenced by a mean study intake (average of midpoint and endpoint) of 1297 mg; while the low calcium group reported a mean study intake of 469 mg. Caloric intake significantly decreased over time in both groups ($p \leq 0.05$) without group differences. The mean calorie reduction from baseline was – 416 kcal (LC) and -225 (HC). Although mean study percent calories from protein was significant between groups ($p \leq 0.05$), protein per kilogram body weight remained constant 0.9 ± 0.1 g/kg LC versus 1.0 ± 0.2 g/kg HC ($p=0.18$) throughout the study.

Strength and exercise compliance

Exercise compliance in the LC group was 93.3% versus 90.9% in the HC group ($p=0.09$). Total workload (load x repetitions) significantly increased over time in both groups ($p \leq 0.0001$) without group differences ($p = 0.16$). Strength increases in both groups were significant in the bench press, squat, deadlift, and dumbbell row exercises ($p \leq 0.001$) (**Table 4**) with no group differences. Percent strength increases from baseline in the major exercises were as follows: bench press 58% LC versus 54% HC, squat 109% LC versus 115% HC, deadlift 131% LC versus 142% HC, and dumbbell row 81% LC versus 78% HC.

Discussion

In comparison to the limited number of clinical trials, [18-19] this study is the first to examine the dairy calcium and weight/fat loss hypothesis in overweight women undergoing 16 weeks of structured resistance training while reducing caloric intake. In this study, high calcium intake did not enhance weight loss when added to structured exercise and modest caloric reduction. This finding is consistent with previous studies. [15, 26] It is important to note that usual calcium intake of participants in this trial was 534 mg and ideal for testing this hypothesis compared to other studies with higher usual intakes of $\geq$ 700 mg. [15, 26] The overall weight loss in this trial for the total sample was modest at 1.9 kg and was primarily the result of a prescribed modest calorie deficit. Intervention calorie intake did not significantly differ between groups, however, the LC group experienced a much larger calorie deficit from baseline to midpoint than the HC group and this likely contributed to the trend in greater weight loss and body composition

changes seen in the LC group. Resistance training likely contributed to small increases in calorie expenditure for all participants but it was unlikely to have added to the observed weight loss outcomes for several reasons: 1.) Total work between calcium groups were similar, 2.) Lean body mass accretion did not differ between groups and likely contributed to any increase in metabolic rate similarly, and 3) Resistance training, in well controlled clinical trials has not been shown to enhance weight loss. [27]

Other clinical trials have prescribed larger calorie deficits of at least 500 kcals [11-16, 28] and have produced much larger weight losses. In contrast, our data shows no added benefit of a high dairy calcium diet on weight loss with a modest caloric restriction. Our weight loss findings between low and high calcium groups are comparable to a recent 8 week resistance training study [19] and a 6 month weight maintenance trial [12] that did not focus on significant calorie reduction.

A major finding in this study is that average total fat loss, trunk fat, sagittal diameter, waist circumference, percent fat, and fat mass index all significantly decreased over the 16 week intervention ($p \leq 0.05$). Furthermore, much of the fat lost was in the truncal region, an area strongly implicated in the development of metabolic syndrome. We found that participants with the largest waist circumference at baseline ($\geq$ 88 cm) trended toward losing more waist circumference than participants with a smaller waist circumference at baseline (< 88 cm). We did not observe group differences in any of the fat indices. However, when race was a covariate, the low calcium group experienced significantly greater reductions in waist circumference and percent body fat. At first glance, this could be explained by the unequal distribution of race between calcium

groups. This is likely not the case considering that African Americans by group (n=10 HC versus n=5 LC) in the study lost -4.7 cm in waist circumference and -3.1% body fat compared to -0.3 cm and -2.2% in non-African Americans (n=3 HC versus n=8 LC). The group differences observed are likely explained by large within group variability in waist circumference and % body fat and perhaps, although not significant, larger mean calorie deficits from baseline observed in the LC group (-416 kcals) compared to HC (-225 kcals). An additional explanation for the larger waist circumference reduction seen in the LC group may be due to the higher number of participants at baseline categorized with a high risk waist circumference (≥ 88cm) (LC, n =6; HC, n=4).

Calcium intake was not correlated with fat mass reduction in this trial. Despite the ambiguous effect of calcium intake on promoting fat reduction, the magnitude of fat loss generated by this intervention is comparable to other studies. [17-19] In contrast, lean body mass significantly increased approximately 1 kg over time in the total sample without observed group differences. This occurred despite relatively low reported study calorie (20 kcal/kg) and protein (0.97 g/kg) intake. In a recent trial, high dairy calcium intake has been associated with lean body mass preservation during weight loss.[12] There was no evidence that high dairy calcium exerted this effect over the course of this trial.

Mean calorie intake significantly decreased over time in both calcium groups. The low calorie per kg midpoint and endpoint values suggest dietary underreporting. This phenomenon should be expected to some degree because the same investigator, who taught the study diets, also collected the food recalls. In addition, underreporting has been observed in women more than men and there appears to be an inverse relationship in the

magnitude of underreporting and body mass index in women.[29] Moreover, a study of women with similar characteristics of participants in this trial examined telephone administered multiple-pass recall methods combined with doubly labeled water and found a 16% rate of underreporting. [30]

We were able to keep protein per kilogram body weight constant over the course of the study. This is important because previous trials have reported greater weight and fat loss with higher protein diets. [28] Although percent energy from protein increased over time, when standardized for body weight, no group differences in protein intake were observed. Vitamin D intake is important to measure in this trial due to its effect on facilitating intestinal calcium absorption and altering other aspects of calcium metabolism. Mean vitamin D intakes did not reach the RDA (5.0 mcg) in the LC group while vitamin D intake in the HC group was 7.25 mcg. Any deficiencies in vitamin D intake were theoretically corrected with vitamin D supplements provided in the trial. Although total carbohydrate intake was significantly different between groups, the differences observed were highly variable and not consistently different between midpoint and endpoint measures and did not appear to be biologically significant.

Strength significantly increased over time in both groups without group differences. Currently there is no known theoretical basis for expecting increased strength from low or high calcium intake. However, other dietary factors such as protein and calorie intake may be affected by dairy intake and therefore, may play a role if group differences in these nutrients are observed. Total work output and workout compliance

were similar between groups and did not contribute to calcium group outcomes of strength and body composition.

Strengths of this study include participant commitment as evidenced by high study exercise compliance (≥ 90%), high total work output per unit of time, and low study dropout rate. In addition, participants were able to make significant body composition changes by exercising 25-30 minutes three times per week and making small healthful dietary changes. Limitations of this trial include probable underreporting of dietary intake as suggested by previous literature and the low reported kcal intake compared to weight loss outcomes. An additional limitation includes not stratifying by race during randomization.

Future research examining the dairy calcium and fat loss hypothesis in women should consider combining dietary intake assessment with doubly labeled water to address concerns of dietary underreporting. In addition, future research designed to examine dairy's role in fat and weight reduction in populations at risk for weight gain should consider longer resistance exercise interventions (> 16 weeks) combined with innovative diet plans designed to examine meal intake timing on promoting changes in resting metabolic rate and body composition.

In conclusion, weight change, fat reduction and strength gains over time are primarily related to the resistance exercise stimulus and calorie reduction and are not enhanced by calcium intake. High calcium diets do not appear to enhance fat mass reduction when combined with chronic resistance training in this population of women. However, we know that excess weight and truncal fat are associated with the risk for

developing metabolic syndrome [31] and this type of program may be a factor in preventing or delaying its onset. This is evident by the weight loss and significant trunk fat reductions experienced in this trial despite relatively small dietary changes and resistance exercise lasting approximately 25 minutes three times per week. Therefore, the convenient and time-efficient nature of this diet and exercise program may be relevant in improving the health of women at risk for further weight gain, obesity and the development of metabolic syndrome.

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Figure 1 Randomization

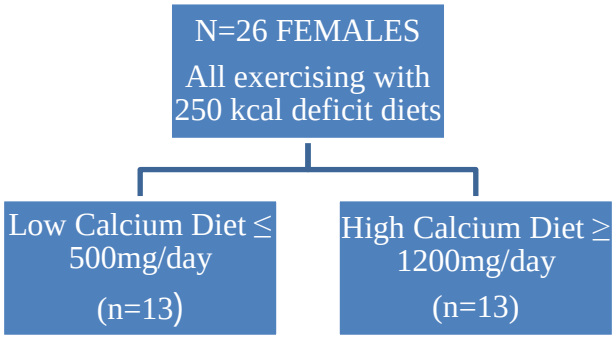

Figure 2 Percent Fat Loss between Calcium Groups

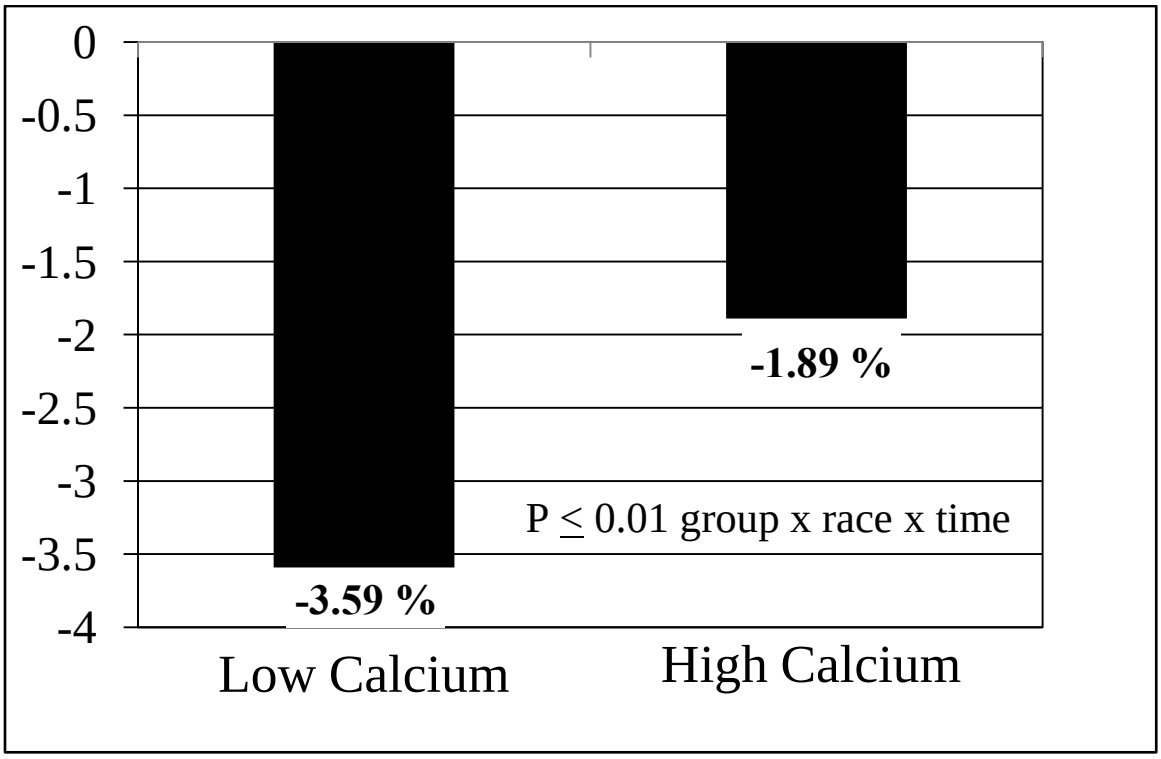

Figure 3 Waist Circumference Loss between Calcium Groups

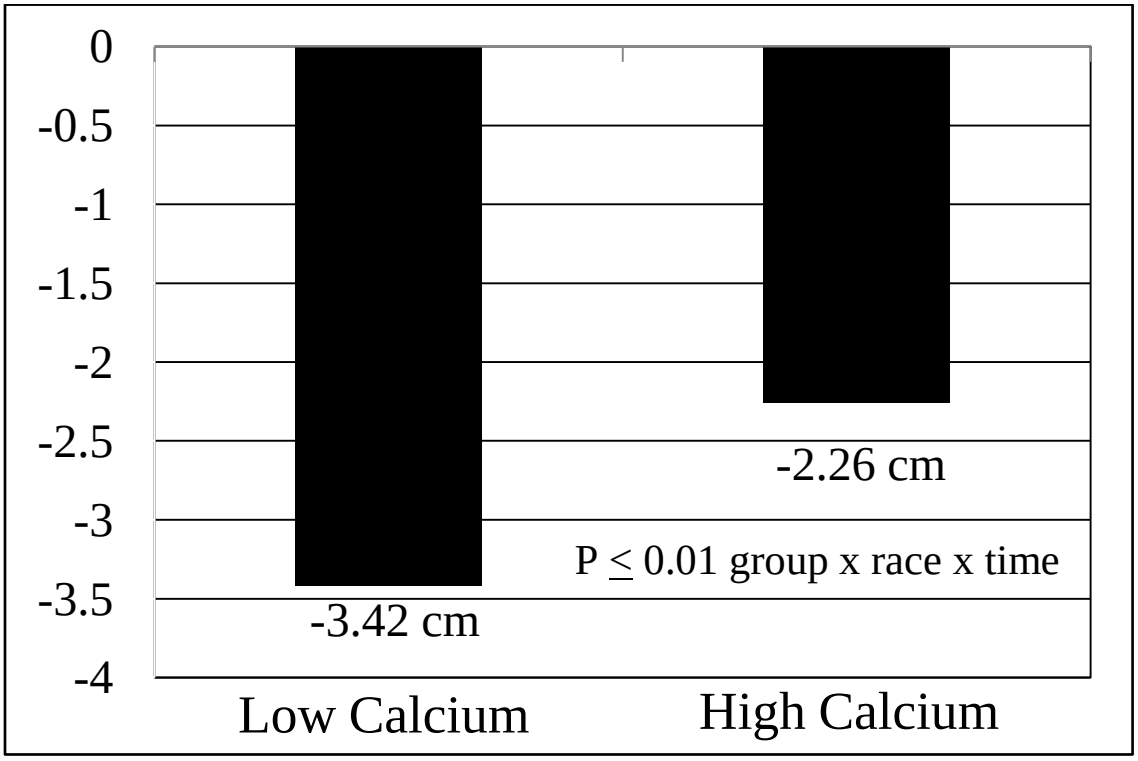

Table 1: Baseline Characteristics of Participants by Group		
	Low Calcium (n =13)	High Calcium (n=13)
Age (y)	37.3 (5.4)	35.9 (4.1)
Weight (kg)	76.2 (7.7)	77.2 (7.1)
Height (cm)	162.7 (6.1)	162.7 (5.6)
BMI (kg/m2)	28.9 (2.5)	29.3 (1.9)
Race (n)[1]		
White	5	3
Black	5	10
Asian	2	0
Latina	1	0
Mean (SD)	[1]Significantly different at $p < 0.05$ χ^2 analysis	

Table 2: Body Composition Measurements at Baseline and Endpoint by Group

	Low Calcium (n = 13)			High Calcium (n = 13)	
	Baseline	Endpoint		Baseline	Endpoint
Body Weight (kg)	76.2 (7.7)	73.8 (7.3)		77.2 (7.1)	75.9 (7.1)
Body Fat %[2]	42.7 (4.1)	39.2 (4.2)		44.2 (3.6)	42.3 (4.4)
Lean (kg)[1]	40.7 (3.2)	41.9 (3.3)		39.9 (4.2)	40.7 (4.4)
Fat Mass (kg)[1]	32.6 (5.6)	29.1(5.4)		34.2 (4.5)	32.2 (5.0)
Fat Mass Index (fat kg/m²)[2]	12.3 (2.1)	11.0 (2.0)		12.9 (1.7)	12.2 (1.8)
Trunk Fat (g)[1]	17429 (3447)	15444 (3625)		16839 (2564)	15522 (2525)
Waist Circumference (cm)[2]	88.4 (6.1)	85.0 (5.7)		84.6 (1.6)	82.3 (5.7)
Sagittal Diameter (cm)[1]	27.1 (1.7)	25.8 (2.1)		25.6 (2.3)	24.4 (2.3)

Mean (SD) [1] $p \leq 0.05$ x time [2] $p \leq 0.05$ group x race x time RMANOVA

Table 3: Dietary Intake at Baseline, Midpoint, and Endpoint by Group

		Low Calcium n = 13				High Calcium n = 13	
	Baseline	Midpoint	Endpoint		Baseline	Midpoint	Endpoint
Calories[1]	1908 (336)	1460 (456)	1524 (260)		1808 (410)	1651 (316)	1514 (249)
Calories per Kg[1]	25.2 (4.6)	19.9 (6.4)	20.8 (3.7)		23.7 (6.3)	21.6 (4.6)	20.1 (4.0)
Protein (g)	75.5 (19.7)	62 (12.1)	73.6 (11.4)		66.3 (20.3)	76.5 (18.3)	77.7 (16.2)
Protein (%)[1]	15.8 (3.5)	18.4 (3.6)	20.2 (4.4)		14.9 (3.6)	20.0 (6.2)	21.3 (5.5)
Protein per Kg	1.0 (0.28)	0.8 (0.2)	1.0 (0.15)		0.9 (0.33)	1.0 (0.26)	1.0 (0.27)
Fat (g)[1]	73.6 (26.5)	39.5 (19.0)	45.1 (12.0)		72.8 (19.9)	49.0 (17.3)	47.1 (19.5)
Total Carbohydrate (g)[2]	240 (31.3)	197 (29.2)	214 (42.1)		221 (64.0)	230 (43.6)	198 (50.6)
Caffeine (mg)	154 (199)	132 (242)	121 (178)		90 (85)	48 (75)	56 (66)
Fiber (g)	19.8 (7.8)	22.6 (5.7)	20.1 (6.1)		13.3 (4.7)	15.8 (3.5)	13.8 (5.0)

Mean (SD) [1] $p \leq 0.05$ x time [2] $p \leq 0.05$ group x time RMANOVA

Table 3: Dietary Intake at Baseline, Midpoint, and Endpoint by Group							
Vitamin D (mg)[1]	2.2 (1.7)	3.0 (2.7)	2.6 (2.6)		3.7 (3.7)	7.2 (3.2)	7.3 (3.2)
Alcohol (g)[1]	2.3 (6.0)	0.01 (0.02)	0.13 (0.25)		3.9 (7.5)	2.1 (7.4)	1.7 (6.2)
Calcium (mg)[2]	542 (137)	481 (153)	457 (172)		526 (139)	1327 (296)	1267 (190)
Mean (SD) [1] $p \leq 0.05$ x time [2] $p \leq 0.05$ group x time RMANOVA							

Table 4: Strength (Weight Lifted) at Baseline, Midpoint, and Endpoint by Group							
		Low Calcium n = 13				High Calcium n = 13	
	Baseline	Midpoint	Endpoint		Baseline	Midpoint	Endpoint
BenchPress (lb)[1]	47.7 (9.9)	66.4 (12.4)	74.6 (11.6)		52.3 (15.4)	68.9 (15.4)	76.9 (15.5)
Squat (lb)[1]	59.2 (15.5)	91.9 (15.3)	117.3 (20.8)		59.2 (16.2)	97.5 (23.8)	122.9 (26.0)
DeadLift (lb)[1]	57.3 (14.5)	94.2 (16.2)	127.3 (21.8)		58.3 (21.7)	93.3 (20.6)	129.2 (20.3)
Rows (lb)[1]	40.0 (6.8)	59.2 (7.3)	70.0 (9.6)		45.8 (11.5)	65.8 (7.0)	78.1 (13.2)
Mean (SD)	[1] $p \leq 0.001$ x time RMANOVA						

CHAPTER IV

THE EFFECTS OF A DAIRY SUPPLEMENT AND CHRONIC RESISTANCE EXERCISE ON INCREASING LEAN BODY MASS AND PROMOTING IGF HORMONAL CHANGES IN OVERWEIGHT SEDENTARY WOMEN

To be submitted to: Medicine and Science in Sports & Exercise

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Abstract

Previous reports suggest that timed protein ingestion before and after resistance exercise can augment lean body mass as a result of resistance training. The objective of this study was to examine the protein supplement timing hypothesis with yogurt in overweight women engaged in a resistance training program with calorie restriction. Participants (age = 36.6 ± 4.7; African American 57.7%, White 30.8%, 11.5% other) with a BMI of 29.1 ± 2.2 kg/m² were randomized to (YOG)(yogurt supplement; n=13) or control (CONT)(isocaloric sucrose beverage; n=13) supplements to be consumed before and after every training session. Participants completed 16 weeks of whole body resistance training three times per week. All participants received reduced calorie (250 kcal deficit) diets. Six dietary recalls were obtained by a multi-pass approach provided by Nutrition Data System software. Body composition was measured by dual energy x-ray absorptiometry, waist circumference, and sagittal diameter. Strength was measured with 1-repetition maximum (1-RM) and resting IGF-1 and IGFBP3 were measured with ELISA. A mean weight loss of 2.5 ± 4.5 kg (YOG) and 1.2 ± 2.6 kg (CONT) occurred as a result of the program. Both groups significantly decreased caloric intake while maintaining their protein intake. Total lean weight increased over time in both YOG (0.87 ± 1.3 kg) and CONT (1.1 ± 1.0 kg) with a 0.7 ± 1.3 kg/m^2 (YOG) and 1.0 ± 1.0 kg/m^2 (CONT) increase in fat free mass index. Large reductions in total fat (YOG = -3.3 ± 4.1 kg versus CONT = - 2.2 ± 2.1 kg) were observed over time ($p \leq 0.001$). Waist circumference, sagittal diameter, and trunk fat decreased significantly over time ($p \leq 0.01$) without group differences. Strength significantly increased over time. No changes

by time or group were observed in resting hormone levels over the course of the trial. These data suggest that pre/post-yogurt supplementation offer no added benefit in increasing lean indices when combined with resistance training and caloric restriction.

Introduction

The timing of protein ingestion is a key factor influencing muscle synthesis and function.[1] The positive effect of pre-exercise and post-exercise supplementation with protein and carbohydrate has been observed in acute [2-5] and chronic [6-9] trials. Research has also indicated that providing protein-carbohydrate supplementation post-exercise can attenuate muscle degradation [10] and therefore, may further contribute to net muscle protein gains over time.

The results from acute studies provide a framework to suggest that protein and carbohydrate (CHO) given immediately post-resistance exercise augment muscle protein synthesis. [1,3-5, 11-13] This induction of protein synthesis following protein/CHO consumption appears instantaneous and is likely the combined result of increased essential amino acid availability and hyperinsulinemia.[3] In addition, Tipton[2] found that the ingestion of essential amino acids and CHO prior to resistance exercise promoted a greater net protein synthesis compared to post-exercise supplementation. This effect may be explained in part by the supplement contributing additional amino acids to the exercising muscles during exercise induced elevated blood flow.

As a result of chronic trials, it is apparent that protein and carbohydrate consumption before and/or after exercise is important for contributing to protein synthesis, skeletal muscle hypertrophy, and strength gains. [6-7,9] Despite these findings, a research gap is present in understanding the chronic benefit of various pre/post-exercise supplementation strategies in women trying to maximize healthful changes in body composition during weight loss. Many of the previous studies that have sought to

examine this hypothesis have utilized commercial supplements to test their hypotheses in men. These supplements may not be practical day to day recommendations for sedentary women starting a resistance training program and therefore, other options should be considered. Thus, examining the use of practical and readily available food supplementation in conjunction with resistance training may function as a valuable strategy for promoting optimal body composition changes in women.

Studies examining the effects of timed protein-carbohydrate ingestion on promoting hormonal change in women are relatively few. Anabolic hormones such as insulin-like growth factor-1 (IGF-1) may work to modulate muscle physiology as a result of a combined resistance training and supplementation regimen. Kraemer [14] found that when carbohydrate and protein were added in conjunction with resistance training in men, the acute IGF-I response was shown to be consistently higher than in participants taking a placebo. In accordance with these findings, a recent study suggested that protein-carbohydrate supplementation during 26-weeks of resistance training enhanced the IGF hormonal response in an untrained sample of men and women.[15] However, in another study acute hormone concentrations (IGF-I) similarly increased following resistance exercise in trained male participants independent of the administration of a high calorie experimental supplement versus a low calorie control supplement (experimental supplement = 1 g/kg glucose, 0.25 g/kg wheat protein hydrolysate, 0.125 g/kg leucine/phenyalanine). [16] In contrast, significant decreases (20%) in insulin growth factor binding protein-3 (IGFBP3) have been observed in the second half of a 25 week training program in men and women who performed 3 sets of circuit training 3 days per week

without supplementation. [18] Furthermore, when endurance and resistance training were combined and dietary supplementation was added, changes in IGFBP3 over 26 weeks were not observed. [15]

Resistance exercise clearly influences the acute hormonal response of IGF. However, chronic changes in the IGF system are conflicting. It is difficult to generalize the results of these studies based on differences observed in participant characteristics, training history, supplement schemes and exercise protocols. Moreover, little research is available to explain the effect of food protein supplementation on the IGF-1/IGFBP-3 response in overweight resistance training females. It is important to investigate how hormones such as IGF-1 and IGFBP3 respond to dietary manipulations in a well controlled study. This may help to explain increases in strength and lean body mass and better define the resistance exercise-induced anabolic response that promotes favorable body composition changes.

In this study, we provided yogurt as a pre-exercise and post-exercise protein-carbohydrate supplement to further examine the effect of protein supplementation on body composition in overweight women. Our primary objective was to examine the role of timed yogurt supplementation in relation to resistance exercise bouts as an effective means to augment increases in lean body mass and strength. We hypothesized that participants consuming yogurt 20-minutes before and immediately after each resistance exercise bout will have greater increases in muscular strength and lean body mass compared to participants receiving an isocaloric, carbohydrate only placebo. A secondary objective was to determine the influence of yogurt supplementation on the IGF-1 and

IGFBP3 response to resistance training. We hypothesized that participants given yogurt supplementation before and after resistance training will experience larger increases in chronic fasting levels of IGF-1 and IGFBP-3 compared to participants consuming a placebo before and after exercise.

Methods

Participants

Participants were recruited using advertisements distributed across the University of North Carolina at Greensboro (UNCG) campus and in the nearby community (see appendix A). Advertisements stated the purpose of the study, inclusion and exclusion criteria, and benefits of study participation. Potential volunteers were instructed to contact the lead investigator to provide height and weight information for body mass index (BMI) assessment. If the interested volunteer met BMI requirements (BMI 25-30 kg/m^2), a follow up interview was scheduled to assess final eligibility. The follow up interview included two questionnaires (see appendix B-C). The first questionnaire collected information on items such as exercise and medical history. The second questionnaire was a validated screening form designed to assess pre-exercise health status. [19]

Two hundred and forty-two volunteers contacted the lead investigator to take part in the initial BMI screening process. If an overweight BMI was determined, an in depth phone interview was scheduled to determine final eligibility. To meet inclusion criteria, interested volunteers must be 29-45 years of age, deemed appropriate for resistance exercise, and no resistance training in the previous 3 months. The premise for selecting

this age group and BMI range was twofold. First, the incidence of obesity increases with age and women of this age group are at a higher risk for weight gain because of career and family related responsibilities leading to decreased physical activity. Additionally, women of this age group who were already overweight may benefit the most from an intervention designed to improve body composition and therefore, reduce their chances of developing obesity related co-morbidities later in life. Specific exclusion criteria included pregnancy or lactation, reported aversion to dairy products, previous history of orthopedic injury, GI disease, endocrine disorders, or any other medical condition that could compromise the safety of participation or confound study results. Interested volunteers who were on any medications that could confound study results were also excluded, including: steroidal drugs, diuretics, calcium channel blockers, insulin or anti-diabetic agents, synthetic thyroid hormones, and over the counter weight loss supplements. Oral contraceptive use was recorded but not considered as criteria for study exclusion. During the screening process, two hundred and one volunteers were deemed ineligible by the established exclusion criteria: age (n = 23), obesity (n = 82), high calcium intake of greater than 700 mg/day (n = 20), under or normal weight (n = 47), scheduling conflicts and personal reasons (n = 19), medical history (n= 10), and actively engaged in an exercise program (n = 5). Thirty-five participants met all eligibility requirements and were invited to the laboratory for baseline measures. This study was approved by the UNCG Institutional Review Board and all eligible participants gave informed written consent (see appendix D) and had study risks explained to them prior to

engaging in baseline measures. We certify that all institutional and governmental regulations concerning the use of human volunteers were followed during this research.

Overall Design

This study was a randomized trial. After baseline measurements of diet, muscular strength, weight, and body composition, participants were randomly assigned to one of two supplement groups (**Figure 1**): 1) Yogurt 20 minutes prior to exercise and immediately post- exercise (YOG) or 2) Isocaloric placebo given 20 minutes prior to exercise and immediately post- exercise (CONT). Random numbers that corresponded to each of the study groups were generated by statistical software and individually placed in sealed envelopes for group assignment.

Measurements of diet, strength, hormones, and body composition were reassessed at study midpoint and endpoint (see Appendix E). The order of events during measurement days was as follows: A) urine sample (to rule out pregnancy), height (baseline only), weight, waist circumference and sagittal diameter measurements, 1-RM strength assessment, and dual energy X-Ray Absorptiometry (DXA) (Lunar-Prodigy Advance Plus) scanning (baseline and endpoint only). On a second lab day that coincided with the early follicular stage of the menstrual cycle, participants arrived at the lab fasting, during the morning hours to provide resting blood samples. Approximately 15 ml of resting blood was collected from a single antecubital venipuncture. During the course of the trial, all volunteers participated in 3 days per week whole body resistance training for 16 weeks and were instructed to follow a nutritious 250 kcal deficit diet 7 days per week for the course of the supervised sixteen week intervention. All participants were

asked to refrain from participating in additional exercise programs or using dietary supplements during the intervention. The primary goals of this study were to promote muscle mass accretion through pre-exercise and post-exercise supplementation, enhance hormone levels, and support the transition to a healthy body composition. Therefore, a modest weight loss of approximately 0.25 kg/week was our goal and was attempted with a prescribed daily energy deficit of 250 kcal. Participant weight was documented weekly and used as a tool to assess diet compliance (see Appendix F). In addition to monitoring body weight changes, participants met with the study registered dietitian (RD) three times per week prior to the exercise sessions to discuss compliance and address diet related questions.

Supplementation

Participants in the YOG group were asked to consume a 6 oz. serving of fat free yogurt (Yoplait Light Thick and Creamy) containing 100 calories, 20 grams of carbohydrate, 0 grams of fat, and 5 grams of protein 20 minutes prior and immediately following each exercise session. Flavor was determined by participants and substitutions were allowed to prevent taste fatigue. Participants in the CONT group were asked to consume a 6 oz. serving of an isocaloric placebo beverage containing 25 grams of carbohydrate, 0 grams of fat, and 0 grams of protein during the same time frame as the YOG group. The CONT supplement was a sucrose sweetened beverage with the same caloric density as the yogurt. Flavor substitutions of the CONT beverage were also allowed to prevent taste fatigue. All participants received supplementation under close supervision of the study RD. The supplementation protocol was blinded to the exercise

trainers in an effort to control training bias. In addition, all research personnel and participants were not permitted to discuss the CONT or YOG supplements during training sessions to maintain blinding principles. All supplements were provided in identical opaque cups to make specific identification of the supplement difficult for study participants.

Measurements

Diet was assessed using the Nutrition Data System for Research. [21] NDS is a nutrition software system designed to collect and assess 24-hour dietary recall information over the phone. This system utilizes the multiple pass recall method to help improve the validity of dietary data. Two random dietary recalls within the same week occurred prior to protocol start, at midpoint, and at study endpoint.

Following supplement randomization, participants received individualized counseling from the study RD and were instructed on the use of an exchange system diet to guide prescriptions for energy intake. The prescribed diet was based on the American Diabetes and Dietetic Association's (ADA) exchange system [20] and was constructed to control for daily protein consumption (~15% of total calories). The exchange system is a standardized food grouping system that is designed to be easily understood and practiced by followers. This system effectively teaches appropriate serving sizes to control energy and macronutrient intake while also allowing participant autonomy with meal planning. Exchange diets were designed by the study RD during initial counseling sessions to promote a modest energy reduction (-250 kcals) from baseline energy needs. The primary method for accomplishing this mild deficit was encouraging participants to reduce their

sugar and fat intake while attempting to maintain nutritious food variety and appeal. Participants in both groups received complimentary vitamin D supplements (100% RDA) to take daily in an effort to prevent insufficient dietary intake. In addition, since folic acid is known to prevent neural tube defects and that participants were asked to refrain from personal supplement use, we provided folic acid supplements (100% RDA) to take daily. Compliance with diet was monitored and maintained by assessing weekly weights, midpoint dietary recall assessment, and providing the opportunity for weekly question and answer sessions designed to address diet adherence. The caloric intake totals from two random baseline 24-hour food recalls were averaged with calculated energy needs for each participant to determine a baseline calorie need estimate. Energy needs for weight maintenance were calculated using the Food and Nutrition Board's equation for determining energy needs in overweight and obese adult women. [21] Adjustment of total energy expenditure (TEE) for physical activity levels (PAL) were accomplished by multiplying the TEE by the appropriate PAL coefficient (sedentary = 1.0 or low active = 1.16). Initial calorie need estimates were determined by averaging initial energy calculations (TEE X PAL coefficient) with the baseline dietary recall information (see Appendix G). The initial intervention calorie prescription was determined by subtracting 250 kcal to theoretically support a 0.25 kg weight loss per week. During the course of the 16-week trial, if weight loss was not progressing as planned, the study dietitian immediately assessed diet compliance and subsequently decided if additional food exchanges needed to be subtracted from the diet plan to create a greater calorie deficit.

Exercise intervention

Whole body resistance training took place three times per week for the entire 16 week study protocol (see Appendix H). Participants trained on Monday-Wednesday-Friday or Tuesday-Thursday-Saturday based on their convenience. The exercises used were: dumbbell chops (for total body warm-up and core stimulation), followed by dumbbell squats, dumbbell bench press, dumbbell rows, and dumbbell dead-lift. Participants completed all training sessions in the Human Performance Lab at UNCG under the close supervision of trained research personnel.

All participants began the training program by completing a 2-week familiarization period followed by 14 weeks of training progression. During the familiarization period participants completed all exercises with 2 sets of 10 repetitions at 60-70% of their initial 1-RM. In order to provide additional familiarization with the squat exercise, participants performed the exercise (weeks 1-2) using only their body weight with an exercise ball placed against the wall prior to advancing to free weight dumbbell resistance (weeks 3-16). Training advanced to 3 sets per exercise at week three with a goal repetition range of eight to twelve. Rest periods between sets were timed and enforced at 60 seconds. Participant progression followed the classic linear model of periodization as strength improved between 1-RM measuring points. [19] Participants gradually progressed to training at 80-100% of their baseline 1-RM while maintaining a goal repetition range of 8-12. Training load estimates, based on percent 1-RM, were readjusted at protocol midpoint (week 8) when strength was reassessed with an absolute 1-RM. In order to assist in maintaining intensity and appropriate progression between

strength measurements (baseline, midpoint, and endpoint) load adjustments were also based on each participant's ability to stay within the intended goal of eight to twelve repetitions.

Strength was assessed with one repetition max testing (1-RM) at baseline, midpoint and at the end of 16 weeks. Participants began with a warm up of 5-10 repetitions at 40-60% of the participant's perceived capacity for one lift. After a short rest period of 2 minutes, 3-5 repetitions were completed at 60-80% of the participant's perceived capacity for the same lift. Finally, successive 1-RM attempts were performed until failure with the goal of determining the true 1-RM within 3-5 trials. [23] Loads were increased by 2-5 kg for each trial and participants were allowed to rest 3-5 minutes between attempts. Verbal encouragement was given at each attempt to maximize performance.

Anthropometric measurements and body composition

Height was measured without shoes on a stadiometer (Accustat Genentech) at week 0. Weekly body weight was measured by a stationary balance beam scale in exercise clothing without shoes. Changes in total and regional body composition (fat and lean mass from soft tissue) from study baseline to endpoint were assessed by DXA. Primary outcomes measured with DXA included total body mass (kg), total body percent fat, fat mass (kg), fat-free mass (including bone mineral content in kg), total lean (minus bone mineral content in kg), and trunk lean/fat (g). Fat mass index [FMI = fat mass kg ÷ height (m^2)] and fat free mass index [FFMI = fat free mass kg ÷ height (m^2)] were derived from DXA fat mass and fat-free mass respectively. FMI and FFMI allow for a 2-

compartment analysis of body composition changes and are better suited at assessing absolute changes than evaluating percent fat or percent lean. [24] In addition to DXA analysis, waist circumferences (Gulick II tape measure) and sagittal diameter (Rosscraft Campbell Caliper 20) were measured to assess changes in central adiposity over time. Waist circumferences were measured at the narrowest part of the waist per American College of Sports Medicine guidelines at all three study time points. [19]

Hormone Assessment

Morning fasting levels of IGF-I and IGFBP-3 were assessed at study baseline, midpoint and immediately following the 16 week intervention. Participants were asked to complete a 12-hour overnight fast prior to arriving at the lab during the early follicular phase of their menstrual cycle. This phase of the menstrual cycle was chosen to control for estrogen induced changes to the IGF system. Participants gave blood by standard venipuncture procedures from an antecubital vein. For IGF-1/IGFBP3 analysis, a maximum of 15 ml of blood was collected at each timepoint (baseline, midpoint, and endpoint), for a total of 45 ml from each participant during the course of the study. All blood samples were processed by centrifugation and serum removal, and then frozen at -80° C for future analysis. Duplicate samples were assayed for IGF-1 (IDS, Fountain Hills, AZ) and IGFBP-3 (IDS, Fountain Hills, AZ) using enzyme-linked immunosorbant assays (ELISA).

Statistics

Data were analyzed with SPSS (version 15.0). Baseline characteristics were compared between groups with Student's t-test or Chi-square analysis. Differences in soft

tissue body composition, weight, anthropometrics, strength, and hormones over time and between groups were determined by repeated measures analyses (RMANOVA). Significance was determined at $p \leq 0.05$.

Results

After randomization, six participants withdrew from the study prior to completing the16 week protocol due to pregnancy (n = 1, YOG), scheduling conflicts (n = 1, CONT), and personal reasons (n = 2, YOG; n = 2, CONT). A total of 29 participants are scheduled to complete the 16 week intervention. The data presented are preliminary and represent a sample size of n = 26.

Baseline Values

There were no significant differences in baseline age, weight, height, or BMI (**Table 1**). No significant differences in race distribution were observed among supplement groups when race groups were dichotomized to either Black (n=8 YOG; n=7 CONT) or Other (White, Asian, or Latina) (n=5 YOG; n=6 CONT). Participant fat free mass index (**Table 2**), calorie and protein intake per kilogram body weight (**Table 3**) were not significantly different between supplement groups at baseline. Measures of strength (1-RM) were all similar between groups at baseline (**Table 4)**.

Body Composition

Changes in body composition by supplement group are outlined in **Figure 2 & Table 2**. Total lean weight increased over time in both the YOG (0.87 ± 1.3 kg) and CONT (1.1 ± 1.0 kg). Participants gained 2.9% (YOG) and 2.4% (CONT) of lean body mass as a result of the intervention with a 0.7 ± 1.3 kg/m^2 (YOG) and 1.0 ± 1.0 kg/m^2

(CONT) increase in fat free mass index. This accretion of lean tissue occurred despite a total mean weight loss of 2.5 ± 4.5 kg (YOG) and 1.2 ± 2.6 kg (CONT) in each supplement group. Significant increases in total appendicular lean tissue were observed over time in both the YOG (251 ± 597 g) and the CONT (339 ± 718 g) without group differences (p=0.74). The same trend was observed with changes in trunk lean tissue for both groups (YOG = 609 ± 1065 g versus CONT = 776 ± 463 g) without group differences (p = 0.63). Large reductions in total fat (YOG = -3.3 ± 4.1 kg versus CONT = - 2.2 ± 2.1 kg) were observed over time ($p \leq 0.001$) but not by supplement group. No group differences were observed in regional fat loss, however both groups reduced their waist circumference (YOG = -2.6 ± 4.5 cm versus CONT = 3.1 ± 3.9 cm), sagittal diameter (YOG = -1.2 ± 1.4 cm versus 1.3 ± 0.9 cm), and trunk fat (YOG = -1.86 ± 2.6 kg versus CONT = -1.4 ± 1.4 kg) significantly over time ($p \leq 0.01$).

Dietary Composition

Dietary intake characteristics by group over the course of the study trial are outlined in **Table 3**. Significant reductions in total caloric intake and calorie intake per kilogram body weight were observed ($p \leq 0.01$). The mean calorie reduction from baseline between groups was – 317 kcal (YOG) and -325 kcal (CONT). Although percent calories from protein significantly increased by time ($p \leq 0.001$), mean study protein intake was 75.3g (YOG) versus 69.6 g (CONT) and was not significant between groups (p=0.23). Furthermore, mean study protein per kilogram body weight remained constant 1.0 g/kg (YOG) versus 0.94 g/kg (CONT) (p=0.47).

Strength and Exercise Compliance

Exercise compliance was 91.5% in the YOG group and 96.2% in CONT ($p = 0.44$). Total workload (load x repetitions) significantly increased over time in both groups ($p \leq 0.0001$) without group differences ($p = 0.78$). Strength increases in both groups were significant in the bench press, squat, deadlift, and dumbbell row exercises ($p \leq 0.001$) (**Table 4**) with no group differences. Percent strength increases from baseline in the major exercises were as follows: bench press 51% YOG versus 61% CONT, squat 107% YOG versus 117% CONT, deadlift 116% YOG versus 158% CONT, and dumbbell row 80% YOG versus 79% CONT.

IGF-1

IGF-1 concentration levels by supplement groups at baseline, midpoint and endpoint are presented in **Table 6**. No change in concentration by time ($p = 0.18$) or by group ($p = 0.17$) were observed. Percent concentration change from baseline was 18% (YOG) and -4% (CONT).

IGFBP3

IGFBP3 concentration levels by supplement groups at baseline, midpoint, and endpoint are presented in **Table 5**. No change in concentration by time ($p = 0.56$) or by group ($p = 0.73$) were observed. Percent concentration increase from baseline was 4% (YOG) and 9% (CONT).

Discussion

To our knowledge this is the first study to examine supplement timing in overweight premenopausal women prescribed a reduced calorie diet during chronic

resistance training. Similar trials have not implemented a calorie restriction in an effort to maintain weight or gain weight/lean body mass.[6-7, 25-26] In this study, protein and carbohydrate in the form of yogurt (100 kcals pre-exercise and post-exercise) did not provide an added benefit to lean tissue accretion compared to an isocaloric sucrose control. Our decision to use a dairy product such as yogurt was based on previous research implicating dairy as an effective vehicle for increasing amino acid availability. [13, 18] In this study, lean body mass significantly increased over time with a concomitant decrease in fat mass in both groups. Strength significantly increased over time in both groups without group differences. Total work output and workout compliance were similar between groups and likely did not act as a covariate to observed group strength and lean body mass outcomes. Therefore, it can be inferred that our protein supplement did not provide a large enough stimulus to augment strength increases associated with supplement induced increases in muscle fiber cross sectional area. Despite the absence of group differences, the overall increase in lean tissue accretion from this trial was 1.0 kg and was similar to the 0.8 kg increase observed in a recent study of post-menopausal women.[9] Their intervention group was given approximately 175 kcal of a carbohydrate and protein supplement post-exercise versus approximately 25 kcal in their control. The intervention group increased lean body mass significantly compared to control. The effect of a higher calorie supplement was likely a significant factor in promoting group differences. However, a positive effect of higher caloric loads in supplements is not always replicated in well controlled trials when the ultimate goal is to promote chronic muscle hypertrophy.[26]

Differences in macronutrient composition of the administered supplements may also help explain the presence or absence of group differences. It is conceivable that the carbohydrate from yogurt ingestion immediately following resistance exercise may work synergistically with the protein in yogurt to chronically enhance muscle mass and strength gains. However, carbohydrate ingestion in the CONT beverage immediately following resistance training likely also influenced muscle physiology by increasing glycogen stores [27-28] and decreasing protein degradation. [29] Furthermore, the CONT supplement was likely absorbed faster than the yogurt supplement. The yogurt used in this trial is primarily made up of casein and lactose and therefore, have longer gastric emptying rates and take longer to reach the bloodstream compared to the sucrose found in the CONT beverage. In addition the protein load of 10 g (pre-yogurt and post-yogurt) may be insufficient to promote significant muscle accretion independent of supplement calorie load. The amount of protein and branched chain amino acids needed to promote muscle synthesis may be higher than expected considering a recent study that provided a load of 20 g did not significantly increase muscle fiber hypertrophy in a 12 week trial of elderly participants. [26] In a study of untrained young males,[30] 10 weeks of resistance training and post-exercise isocaloric supplementation found no significant results between control and experimental groups. The study examined a 370 kcal whey protein, EAA, creatine, and carbohydrate beverage compared to an isocaloric carbohydrate only beverage immediately following 4 days/week resistance training. Results from hydrodensitometry assessment revealed only a trend toward increasing fat free mass in the supplement group from baseline (+3.4 kg) versus control (+1.5 kg) ($p=.07$). In

accordance with these findings, Rankin et al.[31] examined post-resistance exercise Gatorade supplementation (5 kcals/ kg) versus isocaloric low-fat chocolate milk on strength and body composition (DXA) in 19 young untrained men. In this trial diet was controlled and training occurred 3 days/week. All study participants increased muscular strength, decreased percent body fat, and increased fat free mass without group differences.

The overall effect of the intervention diet can also play a significant role in body composition changes during chronic studies and may confound benefits of pre/post-exercise supplementation. We found that accretion of significant amounts of lean tissue occurred despite significant caloric intake reductions in both groups. Given that there were no group differences in calorie intake or an observed supplement effect on body composition outcomes, it can be hypothesized that the primary stimulus responsible for promoting body composition changes was the resistance training. Not surprisingly, resistance training has long been implicated in improving body composition and metabolic rate. [32-36] This hypothesis can further be supported by the lack of group differences among other measured dietary factors such as carbohydrate intake and protein per kilogram body weight. Higher daily intake of protein or carbohydrate may provide the benefit of greater muscle accretion and optimal glycogen storage and could influence group differences.

Meal timing can also affect study outcomes by potentially hiding any effects of supplement stimulus on lean body mass. A limitation of this study is that we did not ask participants to refrain from food intake immediately before or after visiting the lab for

their exercise sessions. Previous studies have controlled for this by asking participants to refrain from food intake as long as 2 hours pre-exercise and post-exercise[9] or have scheduled workouts between scheduled meal times to maximize time without additional food consumption.[26] This dilemma will likely continue to be a design concern in future studies and can be argued that it is not practical to refrain from food intake for long periods following exercise. In fact, providing a sports beverage pre-workout and scheduling standardized meals post-workout, [25] may coincide with natural food patterns in participants and provide a more realistic model for testing the true benefit of pre/post-exercise supplementation in real life situations.

IGF-1 is largely secreted by the liver via a growth hormone stimulated pathway and plays a significant role in protein synthesis and muscle hypertrophy.[37] The major binding protein for IGF-1 is IGFBP3 and is responsible for regulating IGF-1 availability and prolonging IGF-1 circulation.[37] Little is known regarding the effect of food supplementation on promoting IGF-1 or IGFBP3 increases in untrained women. Research is warranted to examine the chronic effect of timed food supplementation on the IGF system since commercial supplementation in the acute setting has increased concentrations of IGF-1 and IGFBP3.[38-39]

In this trial, we did not see a significant time or group effect on IGF-1 or IGFBP3 concentrations in previously sedentary or low active women. Untrained women appear to have large acute hormone responses to exercise [40] but much of the research examining the IGF-I and IGFBP3 response to resistance training has used exclusively males[41] or uses a mixed gender sample and does not test for gender effects. The hormonal response seen in

mixed gender samples may not clearly define the true response in untrained women. Furthermore, studies that examine nutrition supplementation combined with resistance training on IGF system changes are limited in number and report inconsistent results. Borst et al.[17] studied men and women and observed a significant 20% decrease in IGFBP3 levels in the second half of a 25 week resistance training, Ballard et al.[15] did not observe chronic changes in IGFBP3 over the course of 26 weeks of training in a male and female sample who participated in a combined endurance training and resistance training program while receiving dietary supplementation. In contrast Kraemer et al.[42] found IGFBP3 concentrations increased significantly in young men, but not older men following a 10 week resistance training protocol consisting of exercise 3 days per week. IGFBP3 response to chronic resistance exercise has also been investigated in untrained versus trained men[43] as well as younger versus older men.[42]

We did not observe a statistically significant supplement group difference to suggest a beneficial role of timed yogurt supplementation on increasing chronic IGF-1 or IGFBP3 levels. However, we did see an 18% increase in YOG group IGF-1 concentrations with a 4% decrease in CONT concentrations. This pattern is similar to the one observed by Ballard et al.[15] and suggests that yogurt supplementation before and after resistance exercise contributed to the observed changes. This is further supported by similar total work and calorie intake between supplement groups. In contrast, we did not observe a similar concomitant increase in IGFBP3 to correspond with YOG IGF-1 concentrations. Given the minimal IGFBP3 response in this trial, the equivocal results in trials that study both genders, and the positive effects of resistance training on IGFBP3

concentrations seen in males, it is possible that the female IGFBP3 response to resistance training and supplementation is minimal compared to males.

Strengths of this study include participant commitment as evidenced by high study exercise compliance (≥ 90%), high total work output per unit of time, and low study dropout rate. In addition, participants were able to make significant body composition changes by exercising 25-30 minutes three times per week and making small healthful dietary changes. Limitations of this trial include underreporting of diet intake as evidenced by reported kcal intake compared to weight loss rates during the trial, and the absence of a metabolically inactive placebo. Future research examining this topic is needed to further elucidate how timing of nutrient intake before and after resistance exercise can maximize healthful body composition changes in previously overweight sedentary females. The research design should focus on a greater caloric pre/post-supplement load combined with both progressive resistance training and greater total calorie reduction.

In summary, significant increases in strength and lean tissue primarily occurred as a result of chronic resistance training and IGF-1/IGFBP3 levels were not significantly influenced by supplement intake over time. Dietary protein/kg body weight remained both constant and modest over time while still supporting a significant increase in lean body mass. The lack of appreciable group differences in lean body mass may be the combined result of low caloric load of the supplementation protocol, absence of a metabolically inactive placebo and not controlling for meal intake before and after exercise. In conclusion, chronic protein supplementation in the form of yogurt does not

enhance lean body mass, promote strength accretion, or alter IGF-1/IGFBP3 levels in overweight women more than an isocaloric carbohydrate supplement when given before and after resistance exercise. However, the weight loss and significant trunk fat reductions experienced in this trial occurred as a result of the prescribed diet and exercise program. The relatively small dietary changes and resistance exercise lasting approximately 25 minutes three times per week significantly improved body composition over time. The convenient and time-efficient nature of this program may be relevant in improving the health of women at risk for further weight gain by preventing obesity and the development of metabolic syndrome.

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Figure 1 Randomization

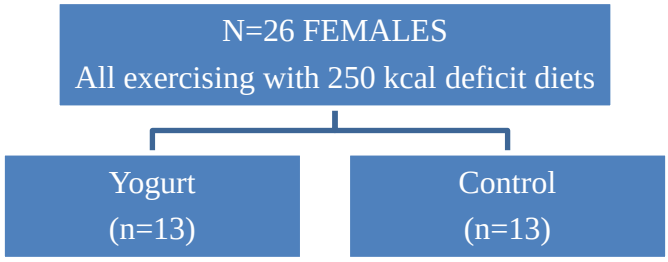

Figure 2 Body Composition Changes by Supplement Group

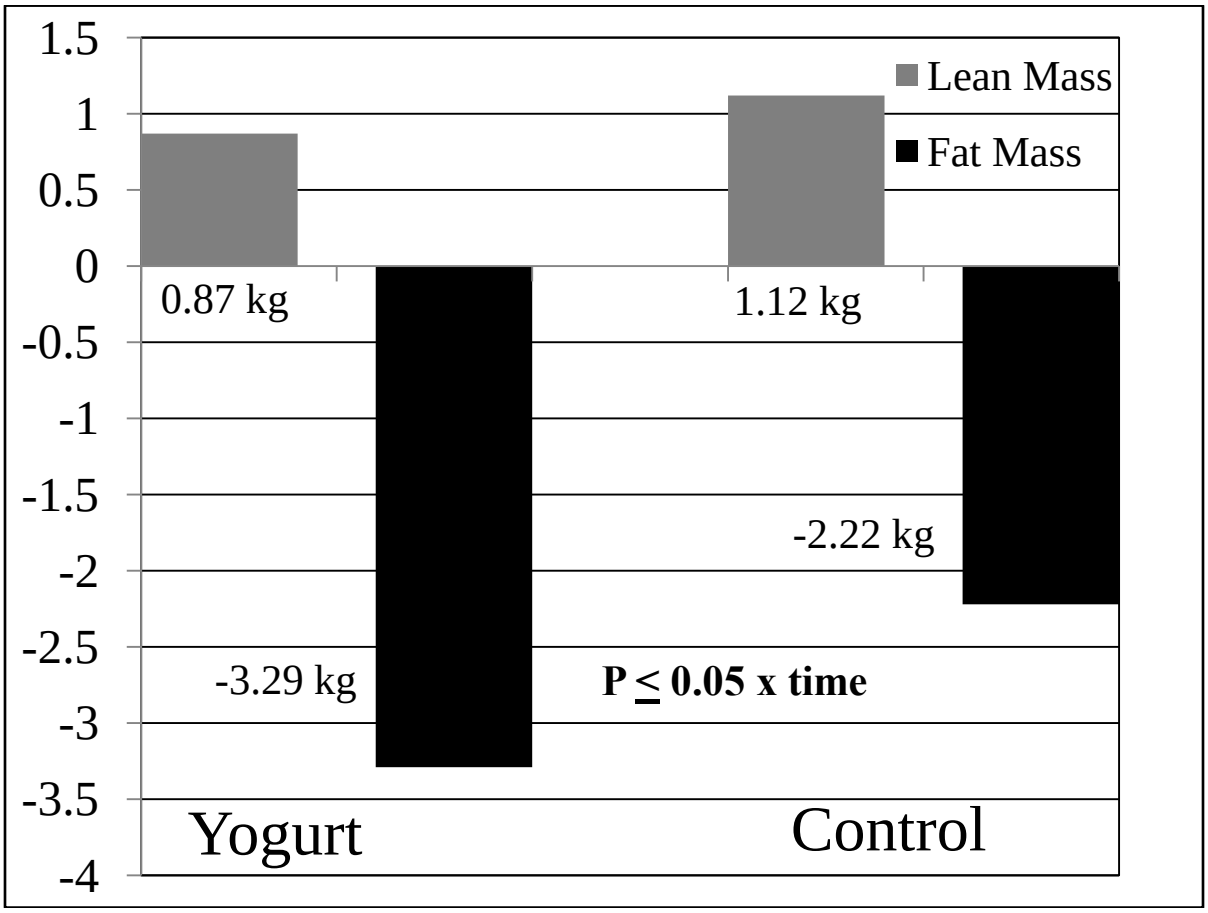

Table 1: Baseline Characteristics of Supplement Groups		
	YOGURT (n = 13)	CONTROL (n = 13)
Age (y)	37.2 (4.8)	35.9 (4.7)
Weight (kg)	78.4 (8.3)	75.0 (5.9)
Height (cm)	163.5 (6.4)	161.9 (5.0)
BMI (kg/m2)	29.4 (2.1)	28.7 (2.3)
Race (n)		
White	3	5
Black	8	7
Asian	2	0
Latina	0	1
Mean (SD)	No significant differences	

Table 2: Body Composition Measurements at Baseline and Endpoint in Supplement Groups

	YOGURT (n = 13)			CONTROL (n = 13)	
	Baseline	Endpoint		Baseline	Endpoint
Body Weight (kg)	78.4 (8.3)	75.9 (7.9)		75.0 (5.9)	73.(6.4)
Body Fat %[1]	43.3 (3.6)	40.3 (4.5)		43.7 (4.3)	41.(4.8)
Body Lean %[1]	56.7 (3.6)	59.6 (4.4)		56.4 (3.9)	58.(4.8)
Lean (kg)[1]	41.4 (4.5)	42.2 (4.8)		39.2 (2.4)	40.(2.5)
Fat Free Mass Index[1] (lean kg/m²)	15.4 (1.1)	15.8 (1.2)		15.0 (0.7)	15.(0.7)
Fat Free Mass (kg)[1]	44.4 (4.8)	45.2 (4.9)		42.2 (2.5)	43.2(2.6)
Fat Mass (kg)[1]	34.0 (5.2)	30.7 (5.3)		32.8 (5.0)	30.(5.6)
Fat Mass Index (fat kg/m²)[1]	12.7 (1.8)	11.5 (1.8)		12.5 (2.1)	11.(2.2)
Appendicular Lean (g)[2]	19680 (2618)	19931 (2624)		18339 (1228)	1867 (1516)
Trunk Lean (g)[1]	18344 (2318)	18953 (2460)		17684 (1361)	18460 (1433)
Trunk Fat (g)[1]	17307 (3129)	15450 (3094)		16961 (2965)	1551 (3153)
Waist Circumference (cm)[1]	85.7 (6.4)	83.2 (7.0)		87.2 (6.0)	84.(5.2)
Sagittal Diameter (cm)[1]	26.5 (1.8)	25.2 (2.3)		26.3 (2.5)	25.(2.3)

Mean (SD) [1] $p \leq 0.01$ x time [2] $p \leq 0.05$ x time RMANOVA

Table 3: Dietary Intake at Baseline, Midpoint, and Endpoint by Supplement Groups

	YOGURT n = 13				CONTROL n = 13		
	Baseline	Midpoint	Endpoint		Baseline	Midpoint	Endpoint
Calories[1]	1819 (476)	1527 (506)	1477 (304)		1897 (237)	1584 (264)	1561 (182)
Calories per Kg[1]	23.5 (7.0)	20.2 (7.1)	19.6 (4.1)		25.4 (3.4)	21.3 (3.6)	21.3 (3.3)
Protein (g)	71.6 (25.2)	71.1 (19.8)	79.6 (16.5)		70.1 (14.5)	67.4 (14.1)	71.7 (9.8)
Protein (%)[1]	15.8 (3.9)	21.3 (5.6)	22.9 (5.7)		15.0 (3.1)	17.2 (3.5)	18.5 (2.7)
Protein per Kg	0.9 (0.4)	0.9 (0.3)	1.1 (0.3)		0.9 (0.2)	0.9 (0.2)	1.0 (0.1)
Fat (g)[1]	73.6 (30.0)	41.2 (19.4)	41.4 (17.0)		72.7 (14.3)	47.2 (17.8)	50.8 (13.8)
Total Carbohydrate (g)	222 (53)	199 (40)	202 (54)		239 (48)	229 (36)	209 (40)
Calcium (mg)[1]	511.6 (141)	929 (451)	895 (434)		557 (132)	879 (542)	829 (480)

Mean (SD) [1]$p \leq 0.01$ x time RMANOVA

Table 4: Strength Measurements at Baseline, Midpoint, and Endpoint by Supplement Groups

	YOGURT n = 13				CONTROL n = 13		
	Baseline	Midpoint	Endpoint		Baseline	Midpoint	Endpoint
Bench Press(lbs)[1]	53.5 (43.9)	68.7 (16.3)	78.1 (15.8)		46.5 (9.9)	66.5 (11.3)	73.5 (10.9)
Squat (lbs)[1]	60.8 (18.0)	99.2 (23.5)	118.9 (25.8)		57.5 (12.9)	89.6 (13.6)	121.3 (20.9)
Dead Lift (lbs)[1]	61.9 (20.0)	96.5 (19.9)	126.5 (24.8)		53.3 (15.0)	90.8 (16.1)	130.0 (60.0)
Rows (lbs)[1]	43.5 (10.5)	61.5 (9.2)	76.5 (14.5)		42.3 (9.3)	63.5 (6.3)	71.5 (8.8)

Mean (SD) [1] $p \leq 0.001$ x time RMANOVA

Table 5: IGFBP3 Concentrations at Baseline, Midpoint, and Endpoint by Supplement Group							
	YOGURT n = 13				CONTROL n = 13		
	Baseline	Midpoint	Endpoint		Baseline	Midpoint	Endpoint
IGFBP3 ng/ml	5810 (1908)	5873 (1975)	6062 (2333)		4849 (1350)	5230 (1585)	5297 (1622)
IGFBP3 = Insulin-like growth factor binding protein 3 Mean (SD) Not significantly different by time or group RMANOVA							

Table 6: IGF-1 Concentrations at Baseline, Midpoint, and Endpoint by Supplement Group							
	YOGURT n = 11				CONTROL n = 12		
	Baseline	Midpoint	Endpoint		Baseline	Midpoint	Endpoint
IGF-1 ng/ml	147.7 (25.1)	182.7 (50.5)	179.0 (41.9)		148.0 (46.3)	150.2 (37.0)	142.3 (43.5)
IGF-1 = Insulin-like growth factor Mean (SD) Not significantly different by time or group RMANOVA							

EPILOGUE

The studies presented in this dissertation suggest that high calcium diets that focus on dairy consumption do not enhance fat or weight loss and the inclusion of yogurt as a pre-exercise and post-exercise supplement does not enhance lean body mass accretion, increase strength, or change anabolic hormone levels. This was observed in a sample of overweight, previously sedentary, premenopausal women who normally consumed≤ 700 mg per day of dietary calcium. In the high calcium study, we surprisingly saw significant reductions in percent body fat and waist circumference in the low calcium group when race was added as a covariate. This is important because most of the previous literature presents either a positive effect of high dairy on reducing fat indices or no group effect at all. Our findings are even more perplexing considering that a disproportional number of African Americans were randomized into the high calcium group. Previous research suggests that African Americans are the racial group most likely to experience enhanced fat loss after adopting a high dairy calcium diet.

The absence of group effects in the supplement study was likely the result of an inadequate protein stimulus found in the yogurt supplement and not controlling for meal intake before and after exercise sessions. Although no group effects were seen, we were not able to measure the beneficial aspects of either of the study supplements without also studying a metabolically inactive (calorie free) placebo. It is possible that all participants gained a metabolic advantage by consuming either study supplement that enhanced their

ability to gain strength and lean tissue from sixteen weeks of resistance training. However, it is important to note that much of the previous literature suggests that post-exercise supplementation including calories from both carbohydrate and protein sources are beneficial and current research is focusing on examining various macronutrient compositions in supplements instead of examining differences in calorically dense versus calorie free supplements. It is also important to note that the types of participants recruited to take part in these studies are not particularly diverse (mainly males) and are interested in improving athletic performance.

As a whole, group differences were not observed when examining calcium intake or supplement intake, however overall changes in body composition and strength were healthful and significant over time. Women in this study significantly increased their strength and lean body mass, decreased overall fat mass, made healthier food choices (based on ADA exchange system diet), and changed from being sedentary to becoming active. Lean body mass and strength increases are important as we age and can lead to many health benefits. Increased metabolic rate is associated with lean mass accretion and contributes to effective long-term weight management. Increased strength improves our ability to perform activities of daily living efficiently while reducing our risk of injury. The reduction of fat indices associated with this program reduces can reduce the risk of developing chronic diseases. Of particular interest is the amount of weight lost in the midsection region as evidenced by significant reductions in both DXA trunk fat and waist circumference. Trunk fat is highly correlated to the development of metabolic syndrome which is characterized by a host of metabolic consequences such as impaired glucose

tolerance, high blood pressure, and a chronic inflammatory state that contributes to the development of chronic disease.

The effects of high dairy diets and food intake timing in overweight sedentary women participating in a chronic exercise programs require further study with novel designs to truly elucidate practical benefits. However, the positive effects of resistance training and small caloric reductions observed in this study are clear. Whole body free-weight resistance training that occurred three times per week and lasted approximately 30 minutes per session (90 minutes per week), effectively contributed to increased strength and positive changes in body composition. Furthermore, the resistance training program design does not require gym membership or expensive equipment and can be considered time-efficient and cost effective. The diet strategy implemented in this program, from a calorie standpoint, was structured to promote a small (-250 kcal) reduction in daily caloric intake. This level of caloric reduction can promote a 0.5 lb (0.25 kg) reduction in weight per week, minimizes feelings of food deprivation, and can easily be achieved by focusing on more healthful food choices (fruits, vegetables, whole grains, low fat dairy). Therefore, this dissertation provides evidence that resistance training and modest daily calorie reduction can be an effective means to improve body composition in overweight women.

Strengths and Limitations

Strengths

1. Few studies have examined supplement timing strategies and the calcium hypothesis in overweight women engaged in chronic resistance training. This is

the first study to examine both of these dietary tactics in overweight premenopausal women participating in a 16 week trial. Therefore, these results contribute to the literature regarding obesity prevention strategies.

2. This study included a diverse sample of overweight women between the ages of 30 and 45 who were previously low active or sedentary. It is conceivable that the positive changes in body composition observed in this program can be repeated in the home setting after a couple weeks of supervised familiarization training. This program is convenient because it does not require gym membership or the promotion of certain “diet” foods. Females in this age range who are busy with family and/or career responsibilities may benefit from this time-efficient diet and exercise program in the comfort of their home with nothing more than a set of adjustable dumbbells.
3. This blood collected in this study provides insight on the magnitude of hormonal changes (IGF-1, IGFBP3) observed in this program. Furthermore, because of the ample amount of serum and plasma collected and stored, several other hormones can be analyzed in the future. This area of research is still young in comparison to research on males and contributes to the literature examining gender differences.
4. Low participant dropout rates and high exercise compliance contributed positively to research outcomes and hopefully can be translated into participant enjoyment of the program.

Limitations

1. Due to the limited research assistant training on the use of NDS and the need for trainer blinding of supplement type, the primary investigator was responsible for both the weekly dietary counseling sessions and the NDS recalls. This likely contributed to significant diet underreporting because of overweight status and the participant desire to please the principle investigator. This is evidenced by low calorie per kilogram estimates. Future studies should consider at least one other person assigned to dietary assessment.
2. The best estimate of self reported dietary intake occurs with 3 random NDS recalls at each study time point. Because of limited participant and principle investigator availability during the intervention, 2 random recalls were completed at each study time point instead of 3. Future work should examine strategies to include 3-4 dietary recalls to improve assessment validity.
3. The variability of weight loss outcomes combined with the relatively small sample size contributed as a limitation in this dissertation. A small number of participants actually gained a significant amount of weight as a result of the program and therefore, affected results over time and by group. The sample size was likely not large enough to negate this effect.

Future Work

At a later date we hope to acquire funding to purchase ELISA kits to assess both chronic growth hormone changes and acute hormone response over time and by supplement group in research participants who completed this trial. These results will

contribute to our knowledge on the magnitude in which growth hormone contributes to the favorable body composition changes observed.

Since the available literature on strategies to promote body composition changes in overweight premenopausal women is still relatively scarce, future research implementing the integration of innovative diet and exercise designs are still needed. I am specifically interested in conducting trials that examine intense and time efficient exercise strategies combined with low calorie nutrient dense meal timing and pre-exercise post-exercise supplementation. These types of trials are important for further investigation into the prevention of weight gain with time efficient strategies. Furthermore, I am interested in examining the benefit of long-term resistance exercise (> 4 months) at varying volumes to and supplementation schemes to contribute to more defined recommendations for the role of resistance training and diet in promoting weight loss/weight maintenance.

Overall, my dissertation research has shed light on the benefit of diet and resistance training on improving the health of overweight women. The results from this study and the valuable experiences gained have personally created a strong professional framework for developing applied research projects in the future designed to tackle the obesity epidemic with innovative diet and exercise strategies.

APPENDIX A: RECRUITMENT FLYER

Are you looking to Lose Fat and Gain Muscle with a Supervised DIET and EXERCISE PROGRAM???

Research Participants Needed!!

Women needed for a research study investigating the effect of diet, food supplementation, and resistance exercise on improving body composition

You may be eligible for this study if you:

- **Are a female between the ages of 30 and 45**
- **Do not currently exercise on a regular basis and consider yourself sedentary**
- **Willing to exercise 3 times per week on the UNCG campus**
- **Meet the study's height/weight requirement (call or email to find out)**

You are *not* eligible for this study if you:

- **Are pregnant or lactating**
- **Have a history of orthopedic injuries of the back, hip, knee, or ankle that limit your exercise ability**
- **Have weight trained regularly in the past 3 months**

The diet and exercise intervention will last 16 weeks. Modest calorie restriction diets will be designed by a Registered Dietitian and exercise will be supervised by trained personnel. Participants will be asked to follow a specific diet and food supplementation pattern. All participants will exercise 3 times per week.

Testing will occur 3 times during the course of the study and will consist of strength, blood and body composition measurements.

Compensation

Each volunteer will receive a supervised exercise plan, free diet analysis, biweekly guidance from a Registered Dietitian, and 3 free body composition analysis profiles during the course of the study.

UNCG

For More Information, Please Contact:

Travis Thomas, MS, RD, LDN
Department of Nutrition
The University of North Carolina at Greensboro
(336) 256-1090
dtthomas@uncg.edu

APPENDIX B: SCREENING FORM

Participant Phone Questionnaire and Screening Form

Part 1: Confirm Initial screening

Participant ID_____
AGE_____

Current Weight_____ Height_____ (*without shoes*)
BMI_____

Part 2: Explanation of Study/Study Essentials

- ✓ to random assignment? _____
- ✓ to supplement? _____
- ✓ diet group? _____
- ✓ to Folic Acid/Vitamin D? _____
- ✓ to exercise 3 times per week at UNCG? _____
- ✓ Three measurement points?_____
- ✓ urine pregnancy tests? _____
- ✓ Blood collection? _____
- ✓ Length of w/o and intervention?_____
- ✓ Upcoming vacations?_____
- ✓ Menstrual cycles?_____
- ✓ No other exercise_____

Part 3: Usual Diet Habits

Typical Breakfast: Typical Lunch:

Typical Dinner:

Daily Beverages/Snacks:
Vitamins/Herbs/Minerals/Supplements: Brand Names:

Fortified Foods:
On an average day, how many servings of dairy (milk, cheese, yogurt) do you consume?
_____

Do you currently have any aversion, intolerance, or allergy to dairy products? If yes, please
explain_____
_____
_____

Part 4: Medical History/Medications

History of Disease or Surgery	***Orthopedic Injury History***
Disease Diagnosis Yes/No	Injury Date/Comments
1) Diabetes/Endocrine Dz	Ankle
2) HTN	Hip
3) GI disease	Knee
4) Kidney disease	Shoulder
5) Heart Disease	Back/Neck
6) Liver Disease	Other Joint Pain
7.) Musculoskeletal	Orthopedic Surgery
8) Surgery	

Para#_____ Time since last birth:_____ wean date:_____

Prescription medications or **birth control** you are currently taking:

Part 5: Physical activity/Exercise History

Are you currently involved in an exercise program? If yes describe_____

_____

_____

Have you ever participated in weight training? _____ If yes, how long ago? _____

Are you still participating in this program? _____

How would you describe your physical activity level?

a.) sedentary (no physical activity) b.) mild physical activity

c.) moderate physical activity d.) very active

Think about the total number of days you are physically active in a given week, Has this activity pattern changed in the past three months? _____, if yes, please explain

_____

_____

_____

Are you currently participating in any sort of **physical activity** or **diet** in hopes of losing/gaining weight or changing body composition?

Part 6: Additional Info/Planning

Reported Ethnicity_____

Menstrual flow start time_____

Starting month_____

Preferred Workout Cycle_____

Preferred Time of Day_____

NOTES_____

_____

_____

_____

_____

APPENDIX C: ACSM SCREENING FORM

AHA/ACSM.Health/fitness facility Preparticipation screening Questionnaire
Assess your health status by marking all *true* statements

History
You have had:
____ a heart attack
____ heart surgery
____ cardiac catheterization
____ coronary angioplasty (PTCA)
____ pacemaker/implantable cardiac
____ defibrillator/rhythm disturbance
____ heart valve disease
____ heart failure
____ heart transplantation
____ congenital heart disease

Symptoms
____ You experience chest discomfort with exertion.
____ You experience unreasonable breathlessness.
____ You experience dizziness, fainting, or blackouts.
____ You take heart medications.

Other health issues
____ You have diabetes.
____ You have asthma or other lung disease.
____ You have burning or cramping sensation in your lower legs when walking short distances.
____ You have musculoskeletal problems that limit your physical activity.
____ You have concerns about the safety of exercise.
____ You take prescription medication(s).
____ You are pregnant.

If you marked any of these statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a **medically qualified staff**.

Cardiovascular risk factors

____ You are a man older than 45 years.

____You are a woman older than 55 years, have had a hysterectomy, or are postmenopausal.

____ You smoke, or quit smoking within the previous 6 months.

____ Your blood pressure is >140/90 mm Hg.

____ You do not know your blood pressure.

____ You take blood pressure medication.

____ Your blood cholesterol level is >200 mg/dL.

____ You do not know your cholesterol level.

____ You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister).

____ You are physically inactive (i.e., you get <30 minutes of physical activity on at least 3 days per week).

____ You are >20 pounds overweight.

____ None of the above

If you marked two or more of the statements in this section you should consult your physician or other appropriate health care provider before engaging in exercise. You might benefit from using a facility with a **professionally qualified exercise staff** to guide your exercise program.

Modified from American College of Sports Medicine and American Heart Association. ACSM/AHA Joint Position Statement: recommendations for cardiovascular screening, staffing, and emergency policies at health/fitness facilities. Med Sci Sports Exerc 1998: 1018.

Professionally qualified exercise staff refers to appropriately trained individuals who possess academic training, practical and clinical knowledge, skills, and abilities commensurate with the credentials defined in Appendix F.

You should be able to exercise safely without consulting your physician or other appropriate health care provider in a self-guided program or almost any facility that meets your exercise program needs.

APPENDIX D: CONSENT FORM

UNIVERSITY OF NORTH CAROLINA AT GREENSBORO

Project Title: The Effect of a High Dairy Diet and Resistance Exercise on Improving Body Composition

Project Director: Cheryl Lovelady Ph.D., R.D., and Travis Thomas, MS, R.D.

Participant's Name: _____

DESCRIPTION AND EXPLANATION OF PROCEDURES:
The purpose of this study is to determine the effectiveness of a high dairy diet along with dairy supplementation before and after resistance exercise on improving body composition when daily calorie intake is reduced. If you consent to participate, the study will begin with an initial analysis of your diet, and measurements of strength, body composition, and hormone levels in your blood. You will be asked to take part in the same measurements at week 8, and week 16 of the study. After initial measurements, you will then be assigned by chance to one of two diet groups to follow for 16 weeks: high dairy calcium or low dairy calcium. You will have assistance from a Registered Dietitian to follow this individualized, healthy, reduced calorie diet. In addition to your assigned diet, you will be asked to consume a supplement 30 minutes before and immediately after weight training three times per week. Study measurements, along with diet and exercise descriptions are outlined below.

All participants of the study will be asked to do the following:

1. Follow an individualized nutritionally balanced diet that will be taught to you by a Registered Dietitian (RD). You will be assigned by chance to the low dairy calcium or high dairy calcium diet. This diet will be easy to follow, allow for food substitutions, and will be constructed to create a small daily calorie reduction.
2. Participate in resistance exercise sessions (45 minutes) three times each week at the Human Performance Laboratory in the Department of Nutrition on the University of North Carolina at Greensboro's campus. A qualified research assistant trained on proper resistance training technique will be present at all of your exercise sessions to provide encouragement, monitor your training technique (to prevent injury), and exercise intensity level.
3. Consume an assigned 6oz. supplement (based on your group) 30 minutes prior and immediately following each exercise session (3 days per week). You will not be asked to consume this supplement on non-exercise days (4 days per week).
4. Participate in 6 short dietary recall interview sessions. You will be called to participate in an interview (twice in one week) prior to beginning the diet and exercise protocol, half-way through the intervention (8 weeks), and at the end of the intervention (16 weeks).

5. This diet record will be used to determine your nutritional intake, develop a reduced calorie diet specifically for you, and ensure compliance.
6. You will be surveyed weekly as part of one of your exercise sessions to notify us of changes in your physical activity or diet outside of the prescribed resistance training/diet intervention. This will also be an opportunity to answer any questions you may have regarding your daily diet and physical activity.
7. You will be weighed once weekly immediately prior to a scheduled resistance exercise session. This weight will be used to assess compliance to the reduced calorie diet.
8. Prior to beginning your diet and exercise routine, at 8 weeks, and at 16 weeks muscular strength will be assessed. We will be testing the strength of your muscles using hand weights.
9. Prior to beginning your diet and exercise routine (baseline) and at 16 weeks you will be given a body scan by dual energy x-ray absorptiometry (DXA). This whole-body scan is necessary to determine your body composition. The scan will be completed at the Human Performance Laboratory in the Department of Nutrition on the University of North Carolina at Greensboro's campus. You will lay still and flat on an x-ray table, and the scanner will move back and forth several feet above you. Depending on your height, the entire procedure takes approximately 30-45 minutes. You will be given a pregnancy test to ensure that you are not pregnant prior to administering the DXA scan.
10. Prior to beginning your diet and exercise routine, at 8 weeks, and at 16 weeks you will be asked to visit the Human Performance laboratory at UNCG twice within one week for measurements. The first visit should take no more than 2 hours (including the DXA scan) and the second visit should take no more than 2 ½ hours (including the exercise session).

    VISIT 1

    a) Urine pregnancy test to rule out pregnancy.
    b) You will be asked to provide approximately 2 teaspoons of venous blood after an overnight fast (no alcohol for 24 hours prior to blood draw). The blood will be drawn in the morning at the lab. Venipuncture will be performed by a trained phlebotomist. The blood is needed to assess your hormone levels.
    c) Trained research personnel will obtain a measurement of your waist circumference using a tape measure. Your weight will also be measured.
    d) DXA scanning (at baseline and week 16 only)
    e) Strength measurements

    VISIT 2

    You will be asked to visit the lab after an overnight fast (no alcohol for 24 hours prior to blood draw) on a regularly scheduled exercise day. You will be given your assigned supplement and be asked to rest quietly for 30 minutes. Right before you begin your exercise session, you will be asked to provide approximately 2 teaspoons of venous blood. Immediately following your exercise you will be given your post-exercise supplement

and will be asked to provide approximately 2 teaspoons of venous blood. Two additional blood samples (2 teaspoons each) will be collected at 30 minutes after exercise, and 60 minutes after exercise.

RISKS AND DISCOMFORTS:
There is a small risk of injury when participating in an exercise program. In addition, temporary muscle fatigue and/or soreness can occur with resistance exercise.
Insertion of the needle during venipuncture may be slightly painful. Every precaution will be taken to minimize the risks involved with venipuncture (air emboli, infection, bruising, and fainting). You will be exposed to very mild radiation from the DXA scan, equivalent to 1/10 the exposure from a routine chest x-ray, and less than the exposure of a dental x-ray.
You may be concerned about bone health if assigned to the low calcium diet group. A 16 week study with low calcium intake is too short to be detrimental to bone health. In addition, the resistance exercise portion of the study provides a protective benefit to bone density.
You may be concerned about the supplementation that you will be asked to consume. All supplements are safe and provide the same amount of energy per serving. In addition, you will be given the opportunity to substitute flavors at your discretion. You may have concerns in regard to providing waist measurements. These measurements will be performed by female research personnel in the privacy of our lab.

POTENTIAL BENEFITS:
Results of all the tests conducted will be provided to you at no cost. Participants in the study will undergo three DXA scans, which provide valuable bone density and body composition information. All participants will receive, at no cost, biweekly nutrition guidance from a Registered Dietitian, free dietary analysis, and a structured supervised exercise program. Benefits to resistance exercising include the potential for increased muscular strength, increased lean muscle tissue and weight loss. Benefits to society include the promoting a healthy weight and preventing further weight gain/obesity.

COMPENSATION/TREATMENT FOR INJURY:
The University has no policy or plan to pay for any injuries you might receive as a result of participating in this research protocol.

By signing this consent form, you agree that you understand the procedures and any risks and benefits involved in this research. You are free to refuse to participate or to withdraw your consent to participate in this research at any time without penalty or prejudice; your participation is entirely voluntary. Your privacy will be protected because you will not be identified by name as a participant in this project.

The University of North Carolina at Greensboro Institutional Review Board, which ensures that research involving people follows federal regulations, has approved the research and this consent form. Questions regarding your rights as a participant in this

project can be answered by calling **Mr. Eric Allen** (research compliance officer) at (336) 256-1482. Questions regarding the research itself will be answered by **Dr. Cheryl Lovelady** or **Travis Thomas** by calling **(336) 256-0310**. Any new information that develops during the project will be provided to you if the information might affect your willingness to continue participation in the project.

By signing this form, you are agreeing to participate in the project described to you by _____.

_____ _____

Participant's Signature Date

_____ _____

Principal Investigator's Signature Date

APPENDIX E: MEASUREMENT DATA SHEET

Strength & Anthropometric Data Documentation Sheet

Participant ID_____ **DATE**_____

MEASUREMENT TIME POINT: Baseline Midpoint Endpoint

EXERCISE DAY: _____/48 WEEK: _____ WEIGHT: lbs kg

BMI:_____ [wt (kg)÷ ht (m^2)] www.nhlbisupport.com/**bmi**

EXERCISE DATA- 1 RM

Exercise	Max Amount Lifted (lbs)	60%	70%	80%	90%
DB Squats					
Bench Press					
DB Row					
Deadlift					

BODY MEASURES

Waist circumference (cm): 1st _____, 2nd _____, 3rd _____, FINAL ☐

Sagittal Diameter (cm): 1st _____, 2nd _____, 3rd _____, FINAL ☐

APPENDIX F: WEEKLY DATA FORM

Weekly Participant Data Collection Form

Participant ID: _____Group ID:_____Week/W/O#_____

1.) Weekly weight (end of week/prior to exercise session): _____

2.) Besides the weight training in this study, how many days in the past week have you participated in vigorous physical activity? (Think about all activities at home, work, or during recreation. Vigorous activity requires heavy breathing and hard physical effort. We are interested in any vigorous activity lasting longer than 10 minutes.)

_____ Days per week → Total time _____

_____ No vigorous activity in previous 7 days

3.) In the past 7 days have you participated in any exercise program other than what was prescribed to you in this study?

APPENDIX G: DIETARY TOOLS

Diet Prescription

Participant ID:_____Date:_____ Time Point:_____

Average NDS intake: Kcals_____

CHO_____

Prot_____

Fat _____

Food and Nutrition Board Maintenance Energy Need Calculation Overweight and Obese Women Ages 19 Years and Older:

TEE= 448 – (7.95 X years) + PA level X (11.4 X kg + 619 X meters)

PA: 1.0/Sedentary; 1.16/Low Active; 1.27/Active; 1.44/Very Active

TEE: _____

NDS/TEE Average:_____

DIET RX:

(-250) Calorie Prescription:_____

CHO exchanges:_____ Percent Kcals CHO _____
Fat exchanges:_____ Percent Kcals Fat _____

Percent Kcals

Protein_____

Diet Group: _____

Research Study Food Group B

You have been randomly selected to follow guidelines for Food Group B. Please refer to the attached list to help with your food selections. Please remember to follow these guidelines everyday (even on the weekends) and to take the provided supplements daily as directed.

Workout Days: (3 days per week)
Please consume at least 2 serving of low-fat milk (8oz) or cheese (1oz) on work out days.

Consume at least 2 servings of non-dairy high calcium foods (≥ *100 mg/serving)*

Non Workout Days: (4 days per week)
Please consume at least 3 servings of low-fat milk (8oz), cheese (1oz), or yogurt on non work out days.

Consume mixed dishes (ex. Casseroles) made with dairy products as desired.

Choose processed and pre-packaged food listing ≥ 20% calcium per serving

Please use the attached list and consume at least 2 servings per day of non-dairy foods that are highest in calcium (≥ 200 mg/serving).

Thank You!

Please contact the study dietitian with any questions:
dtthomas@uncg.edu

Bread, Cereal, Rice, & Pasta Group

Food	Serving Size	Calcium Content (mg)	Calorie Content
Biscuit	1 medium	105	195
Bread, Cornbread	1 slice	110	175
Bread, Spoonbread	1 slice	155	155
Bread, White	1 slice	25	65
-calcium fortified	1 slice	190	75
-calcium fortified, diet	1 slice	180	45
Bread, Whole Wheat	1 slice	20	70
-calcium fortified	1 slice	170	40
Cereal, Total™	1 cup	345	140
Muffin, English	1 whole	100	135
Pancake, homemade	1, 4 inch	110	90
Rolls, Hamburger	1	60	120
Spaghetti, calcium fortified	2/3 cup	300	210
Tortilla, Corn	1, 6 inch	45	60
Waffles	1 medium	20	60

Vegetable Group

Food	Serving Size	Calcium Content (mg)	Calorie Content
Broccoli			
-cooked	½ cup	45	25
-raw	½ cup	20	10
Cabbage, green -cooked	½ cup	25	15
-raw	½ cup	15	10
Cabbage, Bok Choy			
-cooked	½ cup	80	10
-raw	½ cup	35	5
Celery, raw	1 each	15	5
Chard			
-cooked	½ cup	50	20
-raw	½ cup	10	5
Greens, Beet			
-cooked	½ cup	100	15
-raw	½ cup	25	5
Greens, Collard			
-cooked	½ cup	20	25
-raw	½ cup	5	5
Greens, Mustard			
-cooked	½ cup	105	15
-raw	½ cup	40	5
Greens, Turnip			
-cooked	½ cup	100	15
-raw	½ cup	40	5
Kale			
-cooked	½ cup	90	20
-raw	½ cup	50	10

Kohlrabi			
-cooked	½ cup	20	25
-raw	½ cup	15	20
Okra			
-cooked	½ cup	75	30
-raw	½ cup	50	20
Parsley			
-cooked	½ cup	60	15
-raw	½ cup	40	10
Rhubarb			
-cooked	½ cup	235	10
-raw	½ cup	120	5
Rutabaga			
-cooked	½ cup	40	35
-raw	½ cup	30	25
Seaweed Kelp			
-raw	½ cup	70	20
Summer Squash			
-cooked	½ cup	25	20
Watercress			
-cooked	½ cup	65	5
-raw	½ cup	20	2

Fruit Group

Food	Serving Size	Calcium Content (mg)	Calorie Content
Figs, dried	5 each	135	240
Grapefruit			
-half	½ fruit	15	40
-sections	1 cup	30	75
Orange			
-fruit	1 medium	50	60
-juice	1 cup	20	105
-juice, calcium-fortified	1 cup	285	105
-slices	1 cup	70	85

Milk, Yogurt & Cheese Group

Food	Serving Size	Calcium Content (mg)	Calorie Content
Cheese, American			
-fat free	1 oz	200	45
-processed	1 oz	140	105
-reduced fat	1 oz	120	60
Cheese, Cheddar			
-natural	1 oz	150	115
-reduced fat	1 oz	120	50
Cheese, Cottage			
-1% lowfat	½ cup	70	80
-2% reduced fat	½ cup	85	100
-4% fat (regular)	½ cup	60	110
Cheese, Mexican	1 oz	185	105
Cheese, Mozzarella			
-part skim	1 oz	205	80
-whole	1 oz	160	90
Cheese, Muenster			
-natural	1 oz	200	105
-reduced fat	1 oz	205	80
Cheese, Parmesan, grated	2 T	140	45
Cheese, Romano, grated	2 T	140	45
Cheese, Ricotta			
-nonfat	½ cup	200	100
-part skim	½ cup	335	170
-whole	½ cup	255	215
Cheese, Swiss			
-natural	1 oz	270	105
-reduced fat	1 oz	350	90
Ice Cream,			
-regular	½ cup	85	135
-7% fat, light	½ cup	85	110
Ice Milk	½ cup	90	90

Milk			
-skim/nonfat	1 cup	300	85
-1% low-fat	1 cup	300	100
-2% reduced fat	1 cup	295	120
-whole	1 cup	290	150
Milk, Buttermilk			
-skim	1 cup	290	100
-whole	1 cup	275	150
Milk, Chocolate			
-skim/nonfat	1 cup	280	145
-2% reduced fat	1 cup	285	180
-whole	1 cup	280	210
Milk, Dry Powder			
-nonfat	2 T	105	30
-whole	2 T	150	80
Milk, Evaporated			
-skim	½ cup	370	100
-whole	½ cup	330	170
Milk, Goat	1 cup	290	150
Milk, half and half	1 T	15	20
Pudding, ready to eat	½ cup	100	145
Yogurt, fruited			
-nonfat	1 cup	320	160
-2% fat	1 cup	370	250
Yogurt, plain			
-nonfat	1 cup	490	135
-1-2% fat	1 cup	445	155
Yogurt, frozen			
-1% fat	1 cup	175	200

Meat, Poultry, Fish, Dry Beans, Eggs, & Nuts Group

Food	Serving Size	Calcium Content (mg)	Calorie Content
Almonds, raw	2 oz	150	335
Beans, Black, cooked	1 cup	120	245
Beans, Navy, cooked	1 cup	130	260
Beans, Northern, cooked	1 cup	160	245
Beans, Pinto, cooked	1 cup	80	235
Beans, Soy	1 cup	175	300
Chickpeas, cooked	1 cup	80	270
Milk, Soy, canned			
-fat-free (fortified)	1 cup	350	110
-regular	1 cup	10	80
Oysters			
-cooked	3 oz	75	115
-raw	3 oz	40	60
Salmon, canned w/bones	3 oz	180	120
Sardines, canned in water, w/bones	3 oz	65	175
Shrimp, cooked	3 oz	35	85
Tempeh	½ cup	75	165
Textured Vegetable Protein	½ cup	70	185
Tofu Soybean Curd, lowfat, firm	½ cup	30	45

Fats, Oils & Sweets

Food	Serving Size	Calcium Content (mg)	Calorie Content
Molasses, Blackstrap	1 T	175	50

1 cup = 8 fluid ounces; oz = ounce; t = teaspoon; T = tablespoon

APPENDIX H: WEEKLY EXERCISE LOG

Resistance Training Weeks 3-16 Progression **Participant ID:**

3 Days per week 80-100% 1-RM (8-12RM) 3-4 sets per exercise **Please record date and weight lifted each day__60 seconds between sets/exercises

Day 1		DB Chops			Squat			Bench Press			Deadlift			Rows		
Date: W/O #:	Number of Sets															
	Repetition #															
	Pounds Lifted/Set															
Totalwork/exercise																
Day 2		DB Chops			Squat			Bench Press			Deadlift			Rows		
Date: W/O #:	Number of Sets															
	Repetition #															
	Pounds Lifted/Set															
Totalwork/exercise																
Day 3		DB Chops			Squat			Bench Press			Deadlift			Rows		
Date: W/O #:	Number of Sets															
	Repetition #															
	Pounds Lifted/Set															
Totalwork/exercise																
Total Work Lifted/Week																